GENETIC VARIATION AMONG INFLUENZA VIRUSES

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Sequence Variation among Viral Genes

965 CONSERVATION AND VARIATION IN INFLUENZA GENE SEQUENCES, G.M. AIR and R.M. Hall, Australian National University, Canberra.

Sequences of cDNA up to 350 nucleotides long can be obtained by priming from the 3' ends of influenza viral RNA segments.

From the hemagglutinin (HA) gene, this cDNA sequence contains the initiation codon, the coding information for a hydrophobic signal peptide which is removed during maturation of the HA, and the N-terminal part of the mature large polypeptide, HA1. Such sequences have been obtained from genes coding for all 12 of the known subtypes[1] of influenza A HA. They show from 20% to 75% variation in amino acid sequence when the amino acid sequences of the mature HA1 predicted from the nucleotide sequence are compared pairwise. The signal peptides of different subtypes show no common features except the initiating methionine and a high proportion of hydrophobic amino acids.

Hemagglutinin genes within a subtype are surprisingly highly conserved in the N-terminal region of HA1, and although the signal peptide sequences show variation (5 out of 17 amino acids in the most extreme case), these are mostly the result of single nucleotide differences in the cDNA. Between the first (RI/5$^-$/57) and last (Ned/68) members of the H2 subtype, there are 16 nucleotide differences in 320, but only 2/77 amino acid changes in HA1.

To see if drift occurs in genes whose products are not under antigenic pressure, we have obtained cDNA sequences transcribed from the 3' ends of matrix and NS genes (RNA segments 7 and 8) from several human influenza A viruses. During the 23 year period between the isolation of NWS/33 (H1N1) and of RI/5$^-$/57 (H2N2) substitutions have occurred at 7/230 nucleotides in RNA 7 and 13/220 in RNA 8, and in 20 years (RI/5$^-$/75 (H2N2) to CbrGr/77(H3 N2)) substitutions have occurred at 5/230 nucleotides in RNA 7 and 12/220 in RNA 8. These numbers of nucleotide differences are of the same order of magnitude as occurs over a comparable period of drift in the HA genes of the H1 subtype. During drift, therefore, there is not an abnormally high rate of nucleotide change in the HA gene compared with the matrix and NS genes, and from the present data there is no explanation of how the 12 different subtypes of hemagglutinin have arisen.

[1] Bull. W.H.O. Vol. 58 No. 4 (1980)

966 NONVIRAL OLIGONUCLEOTIDES IN CYTOPLASMIC INFLUENZA VIRUS mRNA AS REVEALED FROM CLONED COMPLETED GENOMIC SEQUENCES. Ching-Juh Lai, Lewis J. Markoff, Robert M. Chanock, Michael M. Sveda, and Bor-Chain Lin. Laboratory of Infectious Diseases, NIAID, National Institutes of Health, Bethesda, Md. 20205 USA

We prepared influenza viral DNA duplexes from cDNA copies by reverse-transcription of cytoplasmic viral mRNA and genomic viral RNA. DNA sequences corresponding to gene segments that code for the non-structural proteins, matrix protein, hemagglutinin, neuraminidase, and nucleoprotein of influenza virus [strain A/Udorn/72(H3N2)] were cloned in *E. coli* pBR 322. Analysis of terminal nucleotides of several independently isolated viral DNA segments revealed that additional oligonucleotides were present at the 5'terminus of viral mRNA transcripts. The sequences of these additional nucleotides varied among DNA clones of the same gene and of different genes as well. These sequences also varied in length among clones. The heterogeneity of these oligonucleotides suggests that they were orignally derived from cellular RNA molecules. These results provide evidence that cellular RNA sequences are used to prime influenza viral mRNA transcription.

Sequence analysis also showed that sequences at both termini of vRNA were fully represented in several cloned DNA segments. Thus, these cloned DNA could potentially produce copies of viral RNA which have all the control signals for viral gene expression in eukaryotic cells. As a first step, we attempted to produce influenza viral antigens using the cloned complete DNA sequences. These experiments include: removal of additional dG/dC linker residues that were introduced during plasmid construction, insertion of complete DNA segments into the late region of SV40, and propagation of influenza-SV40 hybrids in the presence of a *ts* SV40 helper in African green monkey kidney cells (AGMK). In one experiment, we observed that infection of AGMK cells with the hybrid virus produced a protein similar to the hemagglutinin of influenza virus as detected by immunoprecipitation and gel electrophoresis. Further studies on viral gene expression will be discussed.

967 GENETIC STRUCTURE OF RNA 8 OF INFLUENZA A VIRUSES

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The smallest RNA segment (RNA 8) of influenza A viruses has been shown to code for at least two viral polypeptides, NS1 and NS2, which possess an approximate length of 230 and 120 amino acids respectively. The NS2 polypeptide is translated from a spliced mRNA whose intervening sequence is contained in the coding region for the NS1 polypeptide. Part of the NS1 and NS2 polypeptides derives from different overlapping reading frames on the RNA. So far, the complete nucleotide sequences of RNA 8 of one avian strain, A/FPV/Rostock/34 (HavlN1), and of two human strains, A/Udorn/72 (H3N2) and A/PR/8/34 (H0N1) have been reported. These RNAs are of the same length, 890 nucleotides; they also share structural features which permit translation of the NS1 and NS2 polypeptides. However, comparison among the three sequences reveals overall differences of 8-9%. The nucleotide differences which are found scattered throughout the genes can all be explained by point mutations.

It is also interesting to note that the negative strands of the three RNAs possess an initiation codon (AUG) at position 98 and that an open reading frame extends to position 598 in the RNAs of A/PR/8/34 and A/FPV/Rostock/34 viruses and to position 745 in the RNA of the A/Udorn/72 strain. Thus, from the nucleotide sequences one can predict polypeptides of 167 amino acids and 216 amino acids respectively. We are now in the process of determining whether this open reading frame on the negative strand of RNA 8 of influenza A viruses is utilized for the synthesis of a third NS gene-specific polypeptide. We are also sequencing RNA 8 of an additional avian strain, A/duck/Alberta/60/76 (Havl0Nav5), to determine the genetic construction of an NS gene which shares little homology with those of the other avian and human strains as determined by DNA-RNA and RNA-RNA hybridization.

968 STRUCTURE, ORIGIN AND FUNCTION OF DEFECTIVE INTERFERING INFLUENZA VIRUS RNAs, Debi Nayak, Alan R. Davis and Rita Cortini, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

von Magnus or defective interfering (DI) influenza virus can be readily produced by passing virus at high multiplicity in either embryonated chicken eggs, chick embryonic fibroblasts, MDBK, MDCK or HeLa cells. Like other DI virus influenza DI virus also requires the helper function of homologous infectious virus, but in turn interferes with the replication of infectious virus. Upon analysis of DI viral RNAs by polyacrylamide gel electrophoresis, DI virus preparation appears to contain, in addition to the eight viral RNA segments, a set of novel smaller RNA segments. These smaller RNA segments appear to be characteristic of a specific DI virus preparation and are responsible for the interference of infectious virus replication. However, unlike other negative strand viruses, namely vesicular stomatitis virus and Sendai virus where the majority of DI virus RNAs appear to arise by a simple deletion of 3' end of viral RNA, DI influenza RNA appears to arise by internal deletion of polymerase (P1, P2 or P3) genes and therefore retains both the 3' and 5' termini of influenza genome. This was shown by oligonucleotide map analysis of DI RNAs and individual vRNA segments and by direct RNA sequencing of 3' and 5' termini of DI RNAs and their progenitors. Recently using a combination of recombinant DNA cloning and RNA sequencing we have completed the sequence of a DI RNA (DI L3) and partially sequenced its progenitor (P1) gene. We find that DI L3 RNA is 443 nucleotides long whereas P1 is approsimately 2500 nucleotides long. After the first 199 nucleotides from the 3' end a deletion of approximately 2050 bp occurs followed by 247 nucleotide sequence at the 5' end of the P1 gene. It appears that the replication event rather than splicing is involved in the generation of influenza DI RNA since the sequence at the deletion point does not resemble that of spliced mRNAs. Additionally, the complementary DI RNA (+) strand contains an open reading frame for 124 amino acids.

DI influenza virus appears to facilitate the establishment of persistent infection (Pi) of MDBK and HeLa cells although DI virus is not later detected in stabel Pi cells. The selection of a slow growing virus variant rather than the presence of DI virus or interferon appears to be responsible for maintaining persistent influenza infection in these cells.

Structure of Viral Genome

969 Antigenic shift and antigenic drift of the haemagglutinin gene in influenza

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The nucleotide sequence of the haemagglutinins of A/Mem/102/72 (H3) and of A/PR8/34 (H1) have been established by cDNA cloning methods.(1)(2) Comparison of the amino acid sequence of different subtypes illustrates that the distinction between antigenic shift and antigenic drift at the molecular level may be somewhat artificial. Comparison of variants within H3 subtypes (3) suggests that the evolution of significant epidemiological variants involves about a 3% amino acid variation within the HA1 subunit. Variation can apparently occur over a wide range of the primary sequence of this subunit. Comparison of the strains A/NT/60/68, A/Mem/102/72 and A/Vic/3/75 (all H3 subtypes) excludes an early theory (4) that drift involves sequential changes of hydrophobic amino acids at a single locus.

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(4) Fazekas de St. Groth, S. (1975) In "Negative Strand Viruses" (B.W.J. Mahy and R.D. Barry, eds.) Vol.2, pp 741-754, Academic Press, London.

970 STRUCTURE AND EXPRESSION OF THE HEMAGGLUTININ GENE OF THE HON1 STRAIN OF INFLUENZA VIRUS, Alan R. Davis, Alan L. Hiti and Debi P. Nayak, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

Using a synthetic DNA primer and reverse transcription we have cloned the hemagglutinin (HA) gene of the A/WSN/33 strain (HON1) of human influenza virus. One clone contains the entire structural gene for HA, thus facilitating studies on the expression of this gene in Escherichia coli. We are now studying this expression. The clone has also been used to determine the nucleotide and protein sequence. The A/WSN HA gene is 1775 nucleotides in length coding for 565 amino acids. The cRNA contains a 5' noncoding region of 31 nucleotides, a coding region of 1695 nucleotides and a 3' noncoding region of 48 nucleotides. The sequence shows a 17 amino acid "signal" prepeptide at the amino terminus followed by HA1 (325 amino acids), a single arginine connecting residue, and HA2 (222 residues) at the carboxy terminus. The comparison with other subtypes reveals many conserved features throughout the primary sequence in all strains and reinforces the argument for a basic architecture required of the hemagglutinin molecule. Comparison of the HO and H2 HAs shows a 67% amino acid sequence homology, which is the highest among the subtypes, suggesting a close geneologic relationship between the two.

971 THE EXPRESSION OF CLONED INFLUENZA GENES IN ESCHERICHIA COLI, J. Spencer Emtage, Department of Molecular Genetics, Searle Research Laboratories, High Wycombe, Bucks, England.

Previous publications for this laboratory have described the molecular cloning of double-stranded copies of Haemagglutinin genes from Influenza Viruses A/FPV and A/Victoria/3/75 (1,2) and the subsequent expression of the Fowl Plague haemagglutinin gene in E. coli (3). This presentation will describe the results of experiments devised a) to increase the expression potential of the FPV gene by using stronger promoters and b) to define in more precise terms the nature of the protein product of the FPV gene.

In virus infected cells, the primary protein product of the haemagglutinin gene is a single polypeptide chain with a number of features characteristic of secreted proteins in general and surface antigens in particular. It contains a pre- or signal peptide at its NH_2-terminus which functions to bind the protein to the endoplasmic reticulum and so ensure its secretion. The haemagglutinin also contains, at its COOH-terminus, a hydrophobic region of 24-26 amino acids which is thought to span the lipid bilayer and so anchor the molecule to the viral envelope. Thus, the production of large quantities of such a protein in E. coli might be expected to present difficulties if, as in the case of preproinsulin (4), the eukaryotic processing signals are recognised by the bacteria and the HA molecules are inserted into the bacterial membrane. We have made constructions of the cloned A/Victoria HA gene lacking these hydrophobic regions to test this proposal and will present the results obtained with them.

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2. W. Min Jou et al. Cell 19, 683-696, 1980.
3. J. S. Emtage et al. Nature 283, 171-174, 1980.
4. K. Talmadge et al. Proc. Natl. Acad. Sci. 77, 3988-3992, 1980.

972 DRIFT AND SHIFT OF INFLUENZA VIRUS STUDIED AT THE GENOMIC LEVEL, W. Fiers, R.-X. Fang, W. Min Jou, D. Huylebroeck, M. Verhoeyen, R. Devos & L. Van Rompuy, Lab. of Molecular Biology, State University Ghent, Ledeganckstraat 35, 9000 Ghent, Belgium.

The HA subtype H3 was present in the human population at the end of the previous century, was identified in ducks and horses in 1963, and caused the Hong Kong pandemic in 1968. Comparison of the HA of A/Victoria/3/75 with A/Aichi/2/68 showed that all changes are due to nucleotide substitutions, except for one triplet insertion. Substitutions are mostly transitions, possibly due to G-U mispairing during replication. Most mutations causing an amino acid change, are selected away, but this selection is less stringent in the HA1-part especially the middle region. Indeed, at a number of sites the mutation avoids immunological inactivation and therefore survives. The net result is an apparently higher variability of the middle region of HA1. Of the 29 amino acid changes between HA Aichi 68 and Victoria 75, we estimated that 16 were involved in antigenic drift (based on changes in HA2 as a neutral clock). Recent data of Wiley, Wilson and Skehel suggest indeed 17 immunologically important changes between these two strains (4 of which are uncertain), divided over four antigenic sites. Remarkably, the implantation of carbohydrate side chains is not completely conserved.

We have also (nearly completely) sequenced the HA gene of A/Duck Ukraine/1963. Compared to Aichi 68 there are no deletions or insertions, but 9.7% nucleotide changes and 4.1% amino acid changes. Taking the silent changes as an internal clock and assuming constant mutation rate independent of host, the data suggest that both originated from a common ancestor gene some 9 to 11 years earlier, i.e. around 1952-1954. This recent divergence strongly suggests an animal HA as the originator of the human H3 pandemic. The evolution 1952-1968 was mostly in the absence of strong immunological selection as the relative accumulation of mutations in HA1 is less pronounced. No framework positions are changed. Among the amino acid positions which are different between Aichi 68, Victoria 75 and Duck 63, 21 are identical between Aichi 68 and Duck 63, while only 5 are identical between Victoria 75 and Duck 63; the antigenic domains C and D are conserved between Duck 63 and Aichi 68. This clearly closer homology between Duck 63 - Victoria 75 constitutes virtual proof that the 1968 pandemic originated by recombining in an H3-type hemagglutinin from an animal reservoir into a human influenza virus.

M. Verhoeyen et al., Nature, 286, 771-776 (1980)

973 CLONING AND DNA SEQUENCE OF DOUBLE STRANDED COPIES OF HAEMAGGLUTININ AND MATRIX GENES OF HUMAN INFLUENZA VIRUS. Mary Jane Gething, Hamish Allen, Jackie Bye, John Skehel* and Michael Waterfield, Imperial Cancer Research Fund, London WC2A 3PX, U.K., and *National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Double stranded copies of the RNA genes coding for the haemagglutinin (HA) and matrix proteins from human influenza viruses have been cloned. DNA sequence analysis provides the complete nucleotide sequence of these genes[1, 2].

The HA gene from strain A/Japan/305/57 (H2 subtype) consists of 1,773 nucleotides containing an uninterrupted coding sequence of 1,686 nucleotides specifying a protein of 562 amino acids. Comparison of the derived amino acid sequence of the HA with those from other human and avian strains reveals the extent of sequence changes in antigenic shifts and drifts. There is considerable homology between all HA sequences with a striking conservation of the location of cysteine residues. This is probably due to constraints on the folding of the molecule and the formation of disulphide bridges. There is also conservation of the number and position of carbohydrate attachment sites although there is some latitude for variation in their location. All HA molecules contain 3 hydrophobic regions. These are the signal sequence at the NH_2-terminus of the precursor molecule, the NH_2-terminal sequence of the HA2 subunit which may have a structural or functional role in viral penetration of the host cell membrane and a sequence at the COOH terminus of HA2 involved in anchoring the HA in the lipid envelope. Although hydrophobicity is conserved in these 3 regions in all HAs, specific amino acid sequence conservation is seen only at the NH_2-terminus of HA2.

The matrix gene from strain A/PR/8/34 is 1027 nucleotides long and contains two open reading frames. These overlap by 68 bases and have a coding capacity for polypeptides of 27,000 and 11,000 daltons. The amino acid sequence derived for the larger protein fits the available data for the matrix protein. The retention of an extended second open reading frame argues for the existence of a second gene product, however there is as yet no evidence that the coding capacity of this gene is used to synthesize a second protein.

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Regulation in Replication, Transcription and Translation

974 BIOSYNTHESIS AND ASSEMBLY OF INFLUENZA VIRUS GLYCOPROTEINS, Richard W. Compans, Michael G. Roth, and Firelli V. Alonso, Department of Microbiology, University of Alabama in Birmingham, Birmingham, AL 35294.

We are investigating the synthesis and intracellular transport of viral glycoproteins in MDCK canine kidney cell monolayers, in which membrane proteins of various enveloped viruses are segregated into distinct plasma membrane domains. In this system, vesicular stomatitis virus (VSV) buds only from the basolateral regions beneath the intercellular tight junctions, whereas influenza virus buds exclusively from the apical surface.[1,2] Although the glycoproteins of these viruses appear to be restricted to the membrane domain from which each virus buds, influenza virus-infected MDCK cells which are superinfected with VSV produce some progeny which appear to be pseudotypes. When the virus yields produced in such a superinfection are assayed under conditions non-permissive for influenza plaque formation, 0.1% to 3% of the VSV plaque-forming particles are resistant to prior treatment with VSV-specific antibody. These presumably represent a subset of phenotypically mixed virions containing influenza glycoproteins surrounding a VSV core; plaques are completely prevented by antibody specific for influenza virus. Such pseudotypes appear in the supernatant medium of doubly infected MDCK monolayers after both the rise of influenza virus production and the first visible cytopathic effects in the monolayer. Prior to this time, no pseudotypes were detected either in the supernatant medium or as cell associated virus. The results indicate that pseudotypes form after destruction of MDCK cell tight junctions; the maturation sites of both viruses remain polarized while the monolayer is intact, and even at later times most of each virus is found at its normal membrane domain.

Monensin, a cationic ionophore, blocks secretion of immunoglobulins from plasma cells and fibronectin and procollagen from fibroblasts. Recently, monensin has been observed to inhibit release of Sindbis and VSV particles into the culture media of baby hamster kidney and chicken embryo fibroblast cells.[3] Monensin treatment of infected cells led to an accumulation within cytoplasmic vesicles of viral glycoproteins which are normally transported to the plasma membrane and subsequently packaged into budding virions. We sought to determine whether the transport of influenza and VSV glycoproteins in MDCK cells can be differentially affected by monensin. Our results show that exposure to a 10^{-6} M concentration of monensin causes a 90% reduction in the yield of VSV, but does not affect influenza virus production, suggesting that the pathways of transport and insertion of these viral glycoproteins into the plasma membrane differ with respect to monensin sensitivity. We are investigating the intracellular location of viral components in monensin-treated cells.

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975 PROCESSING OF THE INFLUENZA VIRUS HEMAGGLUTININ. Hans-Dieter Klenk, Wolfgang Garten, Walter Keil, Heiner Niemann, Ralph T. Schwarz, and Rudolf Rott, Institut für Virologie der Justus-Liebig-Universität, D-6300 Giessen, Germany

The biosynthesis of the influenza virus hemagglutinin, like that of other integral membrane proteins, involves translation at membrane-bound ribosomes, insertion into the membrane of the rough endoplasmic reticulum, and transport to the plasma membrane. In the course of transport, the hemagglutinin undergoes proteolytic cleavage and glycosylation.

Proteolytic cleavage at the posttranslational level converts the precursor HA into the fragments HA_1 and HA_2. It is essential for the capacity of the hemagglutinin to induce membrane fusion and, thus, for virus infectivity. Depending on the host cell, virus particles with cleaved or with uncleaved hemagglutinin may be formed. Comparative sequence analyses of the cleavage site have been carried out on hemagglutinin that has been cleaved either in vivo or in vitro using proteases of various specificities. After in vitro cleavage with trypsin, which is paralleled by activation of infectivity, the N-terminus of HA_2 is identical to that obtained after in vivo cleavage. It differs, however, by a few amino acids from enzymes such as thermolysin or chymotrypsin. These observations suggest that activation of infectivity requires a highly specific amino acid sequence at the N-terminus of HA_2. A basic linking piece appears to be eliminated in the cleavage reaction by the sequential action of a trypsin-like and a carboxypeptidase-B-like enzyme. Evidence has been obtained that the latter activity is associated with virus particles.

Glycosylation is initiated at the rough endoplasmic reticulum by the en bloc transfer of oligosaccharide residues containing mannose and N-acetylglucosamine from a polyisoprenol derivative to the nascent polypeptide chain. After a trimming process, fucose and galactose are attached presumably in the Golgi apparatus. The mature hemagglutinin contains 2 major types of N-glycosidically linked oligosaccharide side chains, the complex type I and the mannose-rich type II. 5 of the 7 potential glycosylation sites of the fowl plague virus hemagglutinin have type I side chains, 1 has a type II side chain, and 1 is not glycosylated. A comparative analysis of 21 influenza A strains containing hemagglutinins of all serotypes known to date revealed distinct differences in the distribution of the oligosaccharide side chains on the hemagglutinin. These observations support the notion that the primary structure of the polypeptide is an important determinant for the carbohydrate moiety of the glycoprotein.

976 OVERLAPPING GENES AND INTERRUPTED mRNAs OF INFLUENZA VIRUS, Robert A. Lamb, Dalius J. Briedis, Purnell W. Choppin and Ching-Juh Lai*, The Rockefeller University, New York, NY 10021 and *L.I.D. N.I.A.I.D., N.I.H. Bethesda, MD 20205

RNA segment 8 of influenza A virus codes for two unique polypeptides, NS_1 and NS_2, which are translated from separate mRNAs. Previous studies[1] on the mapping of these mRNAs on RNA segment 8 using cloned full length DNA (NS DNA) showed that the body of the mRNA for NS_2 was ∿340 nucleotides and the body of the mRNA for NS_1 was ∿860 nucleotides. It was also shown that NS_1 and NS_2 overlapped and that the mRNAs must be translated in different reading frames[1]. We subsequently obtained the complete sequence of the NS DNA and the mRNAs for NS_1 and NS_2. These studies[2] have shown that the NS_2 mRNA contains an interrupted sequence of 473 nucleotides and that the sequences at the junctions of the interrupted segment are similar to the consensus sequences at the splicing sites of intervening regions in eucaryotic mRNAs. The first ∿56 nucleotides at the 5' end of the NS_2 mRNA are the same nucleotides as those found at the 5' end of the NS_1 mRNA, and the leader sequence contains the initiation codon for protein synthesis and coding information for nine amino acids common to NS_1 and NS_2. The ∿340 nucleotide body region of the NS_2 mRNA can be translated in the +1 reading frame, and the sequences indicate that NS_1 and NS_2 overlap by 70 amino acids that are translated from the different reading frames.

We have also found that RNA segment 8 of influenza B virus codes for two unique polypeptides NS_1 (∿40,000 mol.wt.) and NS_2 (∿11,500) which are translated from separate mRNAs. From the size of the polypeptides and RNA segment it can be estimated that NS_1 and NS_2 overlap by ∿120 amino acids.

From the sequence of RNA segment 7 of influenza virus, it has been observed that, in addition to the reading frame coding for the M protein, there is a second open reading frame at the 3' end of the mRNA that could code for a maximum of 97 amino acids[3]. We have searched for a second gene 7 derived product. In hybrid-arrested translation experiments we have found that RNA segment 7 specifically arrests the synthesis of M and a small polypeptide (M_2). This M_2 polypeptide has a tryptic map unique from that of M. Further studies on the characterization of the M_2 polypeptide and mapping its mRNAs will be discussed.

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2. Lamb, R.A. and Lai, C-J: Cell, 21, 475 (1980).
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977 INFLUENZA VIRAL RNA TRANSCRIPTION: THE 5' ENDS OF THE VIRAL MESSENGER RNAS ARE DONATED BY CAPPED CELLULAR RNAS, Robert M. Krug, Stephen J. Plotch, Michele Bouloy, Ismo Ulmanen, Carolyn Herz, Barbara Broni and Ann Beaton, Molecular Biology and Genetics Unit of the Graduate School, Sloan-Kettering Institute, New York, N. Y., 10021.

Capped eukaryotic RNAs act as primers for the synthesis of influenza viral messenger RNA (mRNA) in vitro (and also in vivo) and transfer their 5' terminal methylated cap structure and a short stretch of nucleotides to the viral mRNA (1,2). The stimulation of the initiation of transcription by capped RNAs does not involve hydrogen-bonding between the primer RNA and the virion RNA (vRNA) templates, as capped 5' terminal fragments of mRNAs and capped ribopolymers lacking a sequence complementary to the common 3' end of the vRNA segments (3' UCGUUUUCGUCC) are effective primers (3). Consequently, this stimulation most likely results from a specific interaction between the capped RNA and one or more proteins in the transcriptase complex. This complex, which is associated with purified viral cores, contains an endonuclease that cleaves capped RNAs at a position 10 to 13 nucleotides from the 5' cap, generating capped fragments with 3' terminal hydroxyl groups (4). This unique endonuclease cleaves preferentially at purines (A or G), and requires the presence of a 5' terminal methylated cap structure in the RNA substrate. Most, but not all, of these capped fragments function as primers. Initiation of transcription occurs by the incorporation of a G residue at the 3' end of the capped primer fragment. This G is directed by the 3' penultimate C of the vRNA. Primer fragments with a 3' terminal A residue were more efficiently utilized than those with a 3' terminal G residue, indicating a preference for generating an AGC sequence in the viral mRNA complementary to the 3' end of the vRNA.

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978 INFLUENZA VIRUS-SPECIFIC PROTEINS REGULATING GENOME TRANSCRIPTION AND REPLICATION, B.W.J. Mahy, C.R. Penn, S.T. Nichol and T. Barrett
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The influenza virus genome consists of eight negative single-stranded RNA segments ranging in size from 890 to 2400 nucleotides (1). Transcription and replication of the genome RNA segments is strictly regulated in permissive host cells, as for example during infection of chick embryo fibroblast cells by the avian influenza A virus, fowl plague Rostock (2-4). Using a group of temperature-sensitive (ts) mutants of fowl plague virus (5) we have been able to define certain gene products which are involved in this regulatory process.

The initiation of transcription both in vivo and with purified virions in vitro, is stimulated by capped cellular or viral mRNAs (6). For fowl plague virus, we find that this process requires a functional P2 protein (product of genome RNA segment 1). In vitro transcription by at least five ts mutants, all of which have lesions in RNA segment 1, cannot be primed by globin mRNA at the restrictive temperature. The synthesis of full-length plus-strand template RNA, on the other hand, appears to involve a functional P3 protein (product of genome RNA segment 3). Both this protein, and the P1 protein (product of genome RNA segment 2) may be involved in vRNA synthesis.

Influenza virus induces the synthesis in infected cells of two non-structural (NS) proteins, both of which are products of genome RNA segment 8. Infection with ts mutants indicates that segment 8 gene products are involved in the switch from early secondary to late secondary transcription, and the onset of vRNA synthesis. The non-structural proteins NS, and NS_2 accumulate in the nucleus and cytoplasm respectively in infected cells (7), and possible functional roles of these proteins during replication will be discussed.

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Viral Pathogenesis

979 SUPPRESSOR RECOMBINANTS AND SUPPRESSOR MUTANTS OF INFLUENZA VIRUS AND THEIR BIOLOGICAL SIGNIFICANCE, Christoph Scholtissek and Susan B. Spring, Institut fur Virologie, Justus-Leibig-Universitat Giessen, D-6300 Giessen, Germany

During rescue of temperature-sensitive (ts) mutants of a fowl plague virus (FPV) recombinant with a ts-defect in segment 8 (NS gene) ts^+-recombinants were isolated, which have replaced other segments but not segment 8 carrying the ts-defect. Furthermore, pseudorevertants were isolated, which still carry the gene with the ts-defect. These isolates are called suppressor recombinants or suppressor mutants, respectively, since their genotype is suppressed by recombination or mutation. The suppression also depends on the host cell. Such isolates can be used to identify cooperations between gene products.

980 INFLUENZA VIRUS RECEPTORS ON HOST CELLS-ARE THEY GLYCOLIPIDS? Irene T. Schulze and M. Vijaya Lakshmi, Department of Microbiology, St. Louis University School of Medicine, St. Louis, Missouri, 63104.

It has been clearly established that influenza virions bind to soluble and erythrocyte-associated sialylated glycoproteins via the viral hemagglutinin and that the viral neuraminidase can release the virus from these substrates by removing terminal sialic acid residues from these glycoproteins. However, the relevance of these interactions to the initiation of infection is obscure. Although penetration of the host cell plasma membrane is essential to the infectious process, most of the virions which bind to erythrocytes fail to penetrate. Secondly, although the viral neuraminidase is required for release of virus from these glycoproteins, it is not essential for initiation of infection; virions from which the neuraminidase was quantitatively removed by trypsin were found to be fully infectious so long as the hemagglutinin spikes were not removed[1]. The infectivity of these neuraminidase-free virus preparations was, in fact, increased in the presence of soluble sialylated glycoproteins whereas their hemagglutinating activity was destroyed.[2] Similar effects were observed when sialic acid was covalently attached to galactose residues on the viral hemagglutinin. Again, the infectivity of such preparations was increased and the hemagglutinating activity was decreased although their ability to bind to host cells or to erythrocytes was unchanged.[3] The results indicated that influenza virions could initiate infection when sialylated glycoproteins were bound to the hemagglutinin.

The results described above cast doubt on the assumption that receptors on host cells are like those on erythrocytes and prompted us to attempt isolation of virus receptors from host cell membranes. We have used both binding and plaque assays to investigate the effects of MDBK cell membrane components on the initial interactions of virions with these cells. Using Triton-solubilized cell membranes and affinity chromotography on asialofetuin-sepharose, we isolated a membrane fraction which inhibited influenza virus binding and infectivity but had little effect on vesicular stomatitis virus. This fraction was eluted from the column by galactose. The inhibitory activity was quantitatively recovered in the chloroform following chloroform-methanol extraction. This chloroform-soluble fraction contained trace amounts of a polypeptide which was resistant to degradation by pronase and by trypsin. When the chloroform extract was applied to a florisil column, the inhibitory activity was eluted in the glycolipid fraction. It was not destroyed by proteases, lipase, phospholipases or by a variety of glycosidases, including neuraminidases. It was, however, destroyed by β-galactosidase from bovine liver; this destruction was prevented by galactanolactone, a specific inhibitor of β-galactosidase. The inhibitory component appears to be a glycolipid with terminal galactose residues. The results suggest that, during the initial stages of infection, influenza virions interact with glycolipids in the plasma membranes and that these glycolipids must have terminal galactose residues in order for this interaction to occur. (Supported by NIAID grants AI 10097 and 14590).

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981 GENES INVOLVED IN THE VIRULENCE OF AN AVIAN INFLUENZA VIRUS. A. Sugiura, T. Ogawa, and M. Ueda, The Institute of Public Health, Minato-ku, Tokyo 108, Japan, and National Institute of Animal Health, Yatabe, Ibaraki 505, Japan.

A/chicken/Japan/24(Jap) (Nav1Neq1)[1] is a virus which was isolated from an outbreak of fowl plaque-like illness among chickens and was subsequently found to have the virulence comparable to that of classical fowl plaque virus. Intracerebral inoculation of up to 10 EID_{50} of Jap virus results in paralysis and death of chickens within 3 days. It also kills chick embryos within 48 hours of allantoic inoculation into embryonated eggs. Another avian virus, A/duck/Ukraine/1/63(Ukraine) (Hav7Neq2), in contrast, causes no illness, nor death in either chickens or embryonated eggs even with a large dose. In an attempt to segregate genes of Jap virus responsible for the observed virulence, we prepared recombinants between Jap and Ukraine viruses. Parental derivation of genes in the recombinants was determined by urea-polyacrylamide gel electrophoresis of viral RNA. The virulence was tested by the following three criteria: (1) lethality for 10-day old chickens after intracerebral inoculation, (2) lethality for embryos after allantoic inoculation into 15-day embryonated eggs, and (3) lethality for embryos after allantoic inoculation into 10-day embryonated eggs. The recombinants in which HA, NA, and M genes had been derived from Jap virus and five other genes from Ukraine virus were fully virulent by all three criteria. The recombinants which had received HA and NA genes from Jap virus showed a slightly but definitely diminished virulence toward chickens and 15-day embryos but the virulence for 10-day embryo was unchanged. The recombinants which had received only HA gene from Jap virus had a markedly diminished virulence for chickens and 15-day embryos, causing delayed death in chickens infected with large inoculum dose. They were as lethal for 10-day embryos as Jap virus. It was concluded that HA gene from Jap virus was the key determinant of virulence, but in addition, NA and M genes were required for the full expression of virulence in 15-day embryos and chickens. Lethality for 15-day embryos closely paralleled the virulence for 10-day old chicken not only with the two parental virus strains, but with all recombinants examined, and was found to provide a useful and convenient marker of virulence.

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982 DETERMINANTS FOR THE PATHOGENICITY OF INFLUENZA VIRUSES. R. Rott, H.-D. Klenk, and C. Scholtissek, Institut für Virologie, Justus-Liebig-Universität Giessen, D-6300 Giessen, Germany.

After double infection of cultured cells or an organism recombinant influenza viruses can be obtained caused by reassortment of the viral gene segments. Analysis of a large number of the isolated avian influenza viruses also show that under natural conditions these viruses recombine with high frequency. The recombinants obtained in the laboratory may differ in several parameters from the isolates obtained in the field. Thus among naturally occurring avian influenza viruses the gene which codes for the hemagglutinin clearly is the only determinant for pathogenicity for chicken. Only those viruses are pathogenic the HA-structure of which is susceptible for proteolytic activation in a wide range of host cells. These viruses are capable for rapid multiplication and spread. With recombinant viruses obtained in vitro, however, additional features are required for pathogenicity which are of polygenic nature. This optimal gene constellation which has not been defined yet appears always to be selected for in recombination in nature. With the data available it is proposed what kind of selection pressure may be acting in the formation and maintenance of viruses with optimal gene constellation.

Antigenic Variation and Evolution of Viruses

983 HOST MECHANISMS OF RECOVERY FROM A VIRAL RESPIRATORY TRACT INFECTION: INFLUENZA PNEUMONIA, Francis A. Ennis, Bureau of Biologics, Bethesda, Maryland 20014.

Influenza pneumonia was characterized in Balb/C mice[1] and nude mice bred on a Balb/C background[2]. Balb/C mice had an earlier mean day of death than the nude mice (10 days vs 16 days P <0.01) but eventually more nude mice died (84% vs 58% of Balb/C mice). In addition, virus persisted in the lungs of nude mice (5.8 $\log_{10}$ in nude mice on day 21 vs no detectable virus in Balb/C mice, and lung pathology progressed more slowly but persisted in nude mice. Antibody production was significantly lower and there was no cytotoxic T cell responses detailed in lymphoid cells after infection of nude mice. There was a significant increase in the number of lymphocytes isolated from the lungs of Balb/C mice by day 7 ($t = 5.8$, P <0.001) but not until day 14 ($t = 4.9$, P <0.001) in the nude mice. These results indicate that T cells may be contributing to early deaths in immunocompetent mice, but that T cells were needed for viral clearance and recovery from influenza pneumonia[2]. Passive transfer of normal lymphoid cells to nude and Balb/C mice revealed that radiolabelled lymphocytes preferentially migrated to the lungs of mice with influenza. Passive transfer of lymphocytes from immune mice markedly enhanced the antibody responses of recipient Balb/C and nude mice, but did not afford significant protection to recipient mice. However, transfer of these immune lymphocytes, following exposure to virus antigen in vitro which markedly enhanced their cytotoxic activity did increase recovery from infection and resolution of pneumonia, without enhancing the antibody levels, of recipients. These experiments indicate that cytotoxic T lymphocytes are associated with recovery of mice with influenza pneumonia, but that immune spleen cells which enhanced antibody responses are not.

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984 ANTIGENIC VARIATION IN THE HEMAGGLUTININ OF INFLUENZA A AND B VIRUSES, Robert G. Webster*, William G. Laver**, Michael T. Berton* and Gillian M. Air**, *St. Jude Children's Research Hospital, Memphis, TN 38101 and **Australian National University, Canberra Australia.

Antigenic drift in influenza viruses is believed to occur by selection of variants that are imperfectly neutralized by the host's immune system. Antigenic variants of influenza viruses selected with monoclonal antibodies to the hemagglutinin (HA) molecule show single amino acid substitutions in the large (HA1) polypeptide (1). The frequency of isolation of antigenic variants in influenza A hemagglutinin was approximately 1 in 10^5 but in influenza B it was approximately 1 in 10^8. Studies on A/Mem/1/71 (H3N2) and B/Hong Kong/8/73 influenza viruses with monoclonal antibodies show that there are at least three antigenic areas on the HA molecules. The majority of the antigenic variants of influenza A viruses selected with monoclonal antibodies could not be distinguished by heterogeneous antisera and are probably epidemiologically irrelevant. On the other hand, many of the variants of influenza B virus could be distinguished with heterogeneous antisera and occurred in two of the antigenic areas. The antigenic variants of Mem/1/71 that showed antigenic changes with heterogeneous antisera had single amino acid substitutions at residue 144 or 145. Antisera prepared to these variants contained no detectable specific antibodies to the new determinant and such variants may have a selective advantage in nature.

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985 NATURAL AND UNNATURAL VARIATION IN INFLUENZA A(H1N1) VIRUSES SINCE 1977, Alan P. Kendal, Nancy J. Cox, Centers for Disease Control, Atlanta, GA 30333, Robert G. Webster, St. Jude Children's Research Hospital, Memphis, TN 38101, Paul S. Beare, Harvard Hospital, Salisbury, England, and Setsuko Nakajima, Institute of Public Health, Tokyo, Japan.

In 1977 influenza A(H1N1) virus reappeared into a unique environment; influenza A(H3N2) strains were continuing to circulate, and the majority of the population over the age of 25 years possessed naturally acquired immunity to virus with antigens of H1N1 subtype. The evolution of the virus in this environment has been followed by antigenic, molecular and biological analysis. Observations made include: (1) rapid development of naturally circulating antigenic variants detectable with heterogenous animal sera; (2) more frequent variation of one hemagglutinin epitope (264) than of others detected by testing natural strains with monoclonal antibodies; (3) laboratory confirmation of higher mutability of epitope 264 than other epitopes and 'unnatural' selection of significant antigenic variants with probable point mutation in epitope 264, paralleling natural events; (4) demonstration of mixed infections of individuals with H1N1 and H3N2 strains; (5) identification of 'recombinant H1N1' viruses among natural isolates and cocirculation of these viruses with 'true H1N1' strains and (6) increased or decreased virulence of 'naturally' and 'unnaturally' selected H3N2/H1N1 recombinant viruses. These findings with influenza A(H1N1) virus illustrate the diversity in properties of influenza virus that can rapidly develop by natural or 'unnatural' selection.

986 INFLUENZA: PATHOGENESIS AND HOST DEFENSE, Parker A. Small, Jr., M.D., Reuben Ramphal, M.D., Peter Reuman, M.D., and Richard Kris, Department of Immunology and Medical Microbiology, University of Florida, Gainesville, Florida 32610.

Pathogenesis. Influenza virus apparently destroys ciliated epithelium without the involvement of the immune system, since tracheal desquamation occurs similarly in adult, nude and newborn mice. Severity of influenza illness depends on the initial site of infection and the immune status of the host. In the virgin mouse, initial infection of the lung produces fatal viral pneumonia while initial infection of the nose with up to 30,000 fold more virus leads to a delayed non-fatal viral pneumonia. Nasal infection of heterotypically immune mice does not spread beyond the nose.[1] Post influenzal bacterial pneumonia may result from aspiration of desquamated epithelial cells with adherent bacteria.[2]

Host Defense. Our goal is to understand the role of the different components of the immune system in the prevention of, or recovery from, influenza. Upper respiratory tract (URT) infection must be considered separately from lung infection. Our current understanding is summarized in the following chart:

	Preventive		Recovery	
	URT	Lung	URT	Lung
Systemic Antibody	No[3,4]	Yes[4]	Helps	Helps
Systemic CMI	No[3]	?	Probably	Yes
Local Antibody	Yes[3]	?	?	?
Local CMI	Yes[3]	?	?	?

Prevention of URT infection is mediated by a local immune response and systemic immunity is irrelevant in both ferrets[3] and mice[4]. Prevention of viral pneumonia is mediated by serum antibody.[4] Maternal fetal transfer of antibody also prevents fatal infection and suppresses subsequent antibody production by the infant. Incidentally, this observation casts doubt on the use of acute and convalescent sera for diagnosis of influenza infection of the infant. When this infant mouse has matured it demonstrates immunity in spite of the initial absence of serum antibody. Influenza infections of nude mice demonstrate prolonged viral shedding from both the lungs and the upper respiratory tract. Passive administration of antibody leads to at least temporary regeneration of tracheal epithelium and decreased viral shedding, thus showing that serum antibody can play a role in recovery from influenza.

(Supported by NIH Grant AI07713)

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987 EVOLUTION OF THE HONG KONG HEMAGGLUTININ. STRUCTURAL RELATIONSHIPS BETWEEN Hav 7 AND THE HUMAN H3 HEMAGGLUTININS, Colin W. Ward and Theo A. Dopheide, CSIRO, Division of Protein Chemistry, 343 Royal Parade, Parkville, Victoria, Australia, 3052.

Considerable evidence has accumulated to support the suggestion that genetic re-assortment is one of the mechanisms by which major antigenic shifts can occur in influenza virus. In particular at least one human sub-type, the Hong Kong virus, may have been formed by genetic re-assortment between the existing Asian influenza variant and an animal or bird virus related to either A/equine/Miami/1/63 (Heq 2.Neq 2) or A/duck/Ukraine/1/63 (Hav 7, Neq 2) that had been isolated some five years earlier (1,2).

The relationship between the hemagglutinin from A/duck/Ukraine/1/63 and the human Hong Kong variants has been investigated at the structural level. Amino acid sequence analysis shows that the Hav 7 hemagglutinin closely resembles the human H3 hemagglutinin (3-5). It shows high sequence homology with the Hong Kong hemagglutinin and possesses the characteristic, glycosylated, 10 residue extension at the N-terminal end of HA1.

Various aspects of the Hav 7 sequence will be discussed including its glycosylation pattern and its relationship to the reported structures for several other Hong Kong variants isolated between 1968 and 1977 (3-7).

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Nature of Viral Antigen

988 THE SPECIFICITY OF T CELLS FOR INFLUENZA VIRUS HEMAGGLUTININ
E. Margot Anders, Jacqueline M. Katz, Lorena E. Brown, David C. Jackson, and David O. White, Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia.

T lymphocytes play a central role in the immune response to influenza virus: helper T (T_H) and suppressor T (T_S) cells regulate the antibody response to hemagglutinin (HA), while delayed-type hypersensitivity T (T_D) cells and cytotoxic T (Tc) cells play an important part in recovery from infection. Our studies are directed towards (i) identifying the regions on the HA molecule that represent the antigenic determinants for T cells, in particular T_H, T_S and T_D, and (ii) investigating the way in which T_H or T_S cells of particular specificity influence the final magnitude and quality of the anti-HA antibody response.

In an <u>in vitro</u> assay for T cell help, T_H cells from mice primed with live influenza virus respond to the homologous purified HA, and are also strongly cross-reactive for other HAs of the same subtype as the priming virus. Some evidence for cross-reaction between HA subtypes has also been obtained and is being further investigated. Isolated homologous heavy (HA_1) and light (HA_2) chains are recognised by virus-primed T_H; cyanogen bromide-derived fragments of HA are being used as a finer probe for T_H cell specificity. Similar approaches are being used to study suppressor T cells generated on culture of mouse spleen cells with high concentrations of inactivated influenza virus.

Subcutaneous immunization of mice with inactivated purified influenza virus induces DTH against HA of the priming strain. In this experimental model, DTH has both a subtype-specific and a cross-reactive component. The use of HA_1, HA_2 and cyanogen bromide-derived fragments of HA as eliciting antigen has located T_D determinants on particular identifiable peptides.

989 STRUCTURE AND VARIATION OF ANTIGENIC SITES ON INFLUENZA VIRUS HEMAGGLUTININ, W. Graeme Laver, Gillian M. Air and Robert G. Webster*, Australian National University, Canberra and *St. Jude Children's Research Hospital, Memphis.

During antigenic drift in Type A influenza virus, changes in antigenicity are associated with changes in amino acid sequence of the large hemagglutinin polypeptide, HA1. In 10 variants of Hong Kong (H3N2) influenza virus selected with monoclonal antibodies, the proline at position 143 in HA1 changed to serine, threonine, leucine or histidine. In other variants, asparagine 133 changed to lysine, glycine 144 to aspartic acid and serine 145 to lysine. All these changes are possible by single base changes in the RNA except the last, which requires a double base change. Residues 142-146 also changed in field strains of Hong Kong influenza isolated between 1968 and 1977 (1,2).

In order to determine whether sequential changes at the same position occurred during antigenic drift, antibody prepared against the new antigenic site on the variants in which proline 143 changed to histidine or threonine was used to select second generation variants of these variants. In the first case, the glycine residue (144) next to the histidine changed to aspartic acid and in the second, the threonine at position 143 reverted to proline and the virus regained the antigenicity of wild-type.

Although monoclonal antibodies revealed dramatic antigenic differences between the variants and wild-type virus, only those variants with changes at position 144 of glycine to aspartic acid or at position 145 of serine to lysine could be distinguished from wild-type virus using heterogeneous rabbit or ferret antisera.

These findings suggest that sequence changes in the region comprising residues 142-146 of HA1 affect an important antigenic site on the H3 hemagglutinin molecule, but how these changes affect the antigenic properties, or whether this region actually forms part of the antigenic site is not yet known.

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990 THE 3 ANGSTROM STRUCTURE OF THE 1968 HONG KONG HAEMAGGLUTININ AND IT'S IMPLICATIONS FOR ANTIGENIC VARIATION, Don C. Wiley, Ian A. Wilson (Department of Biochemistry and Molecular Biology, Gibbs Laboratory, Harvard University, Cambridge, Massachusetts 02138) and John Skehel (National Institute of Medical Research, Mill Hill, London NW7).

The 3Å structure of the AICHI/68 haemagglutinin membrane glycoprotein, released by bromelain, reveals an unusual loop-like topology for the polypeptide chain. The chain begins at the membrane end, extends 135Å distally, and returns to end at the membrane. The molecule has a fibrous stem region on top of which a globular region is found. Proteins of the globular region have been identified as antigen binding sites on the basis of amino acid sequence changes in natural and laboratory variants. At least one change in each "antigenic site" appears required for the reoccurrance of disease in a previously infected population.

The oligosaccharide chains are distributed along the length of the molecule with a majority of sites in the half of the molecule nearest the viral membrane. The cleavage site between HA_1 and HA_2 is 100Å from the distal tip of the molecule and 35Å from the viral membrane end. The 21Å separation between termini at that point indicates that a conformational change took place after cleavage. A pocket of conserved residues on the distal end of the molecule is identified as the binding site for the host sialic-acid-containing virus receptor.

991 IMMUNOCHEMICAL PROPERTIES OF INFLUENZA VIRUS HEMAGGLUTININ AND ITS FRAGMENTS., David C. Jackson, Lorena E. Brown, Julie M. Murray and David O. White. Department of Microbiology, University of Melbourne, Parkville, Victoria,3052, Australia.

Several immunochemical approaches have been applied to the characterisation of the antigenic determinants on the hemagglutinin (HA) of influenza virus.

Using various types of radioimmunoassays it has been shown that antigenic activity is carried by two particular peptides derived by cyanogen bromide cleavage of the HA from A/Memphis/102/72. These are HA_1CN1 (the N-terminal 168 amino acid residues of the "heavy chain", HA_1)[1] and HA_2CN1 (the C-terminal 90 amino acid residues of the "light chain". HA_2)[2]. Antibodies raised against HA_1CN1 bind to intact virus and inhibit hemagglutination. One other cyanogen bromide fragment obtained from HA_1 is bound by antibodies raised against isolated HA but not by antibodies raised against intact virus suggesting that this determinant is located in an inaccessible region of the HA spike when present on the virus.[3]

Monoclonal antibodies have also been employed to analyse the number, nature and location of antigenic sites on virus, HA, HA_1 and cyanogen bromide fragments. Competitive binding of these radiolabeled monoclonal antibodies has provided an insight into the topography of distinct antigenic regions on the surface of the molecule.

Solid phase synthesis of a peptide representing a region of HA_1 in which monoclonal antibody induced amino acid substitutions are known to occur has also been carried out. The antigenic and immunogenic properties of this peptide have been tested.

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Prophylaxis, Therapy

992 INFLUENZA IMMUNOPROPHYLAXIS AFTER 30 YEARS EXPERIENCE, Walter R. Dowdle, Office of the Director, Centers for Disease Control, Atlanta, Georgia 30333.

The summary report of the Secretary's Conference on Influenza, July 26, 1978, states that "As a broad generalization, influenza vaccine can be expected to prevent infection and disease for 1-2 years after vaccination in about 7 of 10 people who receive it." The key word is "generalization." After 30 years, questions still persist as to the efficacy of inactivated vaccines under certain conditions and in certain populations for whom the vaccine is recommended. Many questions related to efficacy stem from the inate antigenic and biologic variability of influenza viruses and the variable susceptibility of host populations. Other questions relate to the validity of the vaccine trial itself and the methods used for evaluation of efficacy. Deficiencies in knowledge still remain as to efficacy of inactivated aqueous vaccines in the elderly and the chronically ill and the consequences of annual vaccination.

993 SPECIFIC INHIBITION OF VIRAL REPLICATION BY OLIGOPEPTIDES WITH AMINO ACID SEQUENCES SIMILAR TO THOSE AT THE N-TERMINI OF VIRAL POLYPEPTIDES, P.W. Choppin, C.D. Richardson, M.-C. Hsu, and A. Scheid. The Rockefeller University, New York, N.Y.

Paramyxoviruses and myxoviruses each possess two membrane glycoproteins. The larger paramyxovirus glycoprotein (HN) is responsible for receptor binding (hemagglutinating) and neuraminidase activities, and the smaller (F) is involved in virus penetration through fusion of the viral and cell membranes, and in virus-induced cell fusion and hemolysis. The biological activities of the F protein are activated by proteolytic cleavage by a host enzyme of a precursor (F_o) to yield two disulfide-linked polypeptides (F_1 and F_2). The cleavage generates a new N-terminus on F_1, and the amino acid sequence of this new N-terminal region has been found to be highly hydrophobic and conserved among different paramyxoviruses from various species. A change in conformation and an increase in detergent binding by the F protein accompanies the activating cleavage, findings compatible with an increase in the exposed hydrophobic surface of the protein. In myxoviruses the smaller glycoprotein (NA) is the neuraminidase and the larger (HA) is responsible for both receptor binding and virus penetration. Penetration is activated by proteolytic cleavage of HA to two disulfide-linked polypeptides (HA_1 and HA_2).

The amino acid sequence at the N-terminus generated on HA_2 by cleavage is similar to that of the new N-terminus on the paramyxovirus F_1 polypeptide. Thus there is a structural and functional analogy between the F and HA proteins and their activation. Because several lines of evidence suggested that the N-termini generated by cleavage were involved in the biological activities of the proteins, we hypothesized that it might be possible to inhibit specifically these activities by competitively interfering with this region of the proteins. Oligopeptides were therefore synthesized that resembled the N-terminus of F_1 or F_2, and also a series of related peptides that varied in sequence, steric conformation, and the presence of N-terminal or C-terminal additions. These oligopeptides were tested for inhibitory activity against paramyxoviruses and myxoviruses. Specific oligopeptides were found to be highly effective, and some characteristics of their inhibitory activity are as follows: 1. amino acid sequences similar to those of the F_1 or HA_2 N-termini were required, indicating that inhibition was sequence specific; 2. a carbobenzoxy group or a dansyl group on the N-terminal amino acid increased activity; 3. esterification of the C-terminal amino acid decreased activity; 4. the steric configuration of the first two amino acids affects activity. Specific oligopeptides labeled with radioactive or fluorescent probes have been synthesized and are being used to investigate their precise site and mechanism of action. Such studies should provide further information on this possible new approach to chemical inhibition of viral replication, on the mechanism of action of viral proteins in virus penetration and virus-induced cell fusion and hemolysis, and on membrane fusion in general.

994 BASIS FOR IMMUNITY TO INFLUENZA IN MAN, Robert B. Couch, Julius A. Kasel, Thomas R. Cate, Department of Microbiology & Immunology, Baylor College of Medicine, Houston, Texas 77030.

Infection or vaccination with an influenza virus is accompanied by the induction of a variety of humoral and cellular immune functions. Serum antibody to the hemagglutinin (H) detected by HI or neutralization tests correlates with resistance to infection. Resistance to a heterotypic H3N2 virus among adults with known prior H3N2 infection was related to whether or not they had developed serum antibody reacting with the heterotypic virus. Evidence that serum IgG antibody alone is capable of providing immunity was suggested by the finding of a correlation between cord antibody titer at birth and age at time of infection (1). Described mechanisms for such an antibody mediated resistance are interference with adsorption, aggregation of virions, opsonization for phagocytosis, antibody-complement mediated lysis of infected cells, and antibody dependent cell mediated cytotoxicity.

Serum antibody to the neuraminidase (N), which is capable of ameliorating infection and illness, was also associated with resistance when present in very high titer. Absence of a significant effect of antibody to other virion antigens was suggested by the finding that a clear relationship of serum anti-H antibody to immunity existed even when only purified H and N were used for immunization. A role for anti-H antibody in secretions, particularly for sIgA, has been similarly sought by methods that include use of sensitive radioimmunoassay procedures, and serum IgG antibody remains a more consistent correlate with resistance to infection.

Lymphocyte blastogenesis in response to H1N1 virus did not correlate with resistance to H1N1 challenge, and, although not yet tested in humans, the lack of subtype specificity of T cell cytotoxicity (2) suggests that T cell mediated mechanisms are not likely to be primary mediators of resistance.

Although inadequately studied in children experiencing first infection, the early appearance of multiple humoral and cellular responses during infection that might play a role in recovery makes it unlikely that the relative contribution of each can be assessed in humans.

Thus, although different immune factors and mechanisms that might influence recovery from influenza virus infection and more sensitive techniques for evaluating various humoral and cellular mechanisms of immunity have been described in recent years, serum IgG antibody to the influenza virus hemagglutinin remains the best correlate and most probable mediator of immunity to influenza.

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995 DEVELOPMENT OF COLD RECOMBINANTS OF INFLUENZA VIRUS AS LIVE VIRUS VACCINES. H. F. Maassab, Dan C. DeBorde, Nancy J. Cox and Alan P. Kendal

Development of attenuated live influenza virus vaccine have been pursued in the hope that a relatively asymptomatic respiratory virus infection with such viruses would provide a more effective and durable immunity against influenza virus infection. A suitably attenuated immunogenic and genetically stable "Master Strain", adapted to growth at suboptimal temperatures (25^{0}) and possessing cold-adapted (ca) and temperature-sensitive properties (ts) served as a donor of "attenuated" genes to new wild type strains of epidemiologic relevance to man. The method used for producing live vaccine candidates is recombination-reassortment. Cold recombinants produced were evaluated for attenuation, stability and immunogenicity first in ferrets and then in man. The genealogy of all RNA's in the recombinant was determined so as to provide a basis for understanding which gene(s) from the "Master Strain" responsible for loss of virulence. For reproducibility and speed, laboratory criteria are provided to monitor the genetic basis of attenuation, during vaccine development and during use in man. The studies to be reported suggest that the ca phenotype can consistently indicate satisfactory attenuation in vaccine candidates with six genes derived from the cold-adapted (ca) "Master Strain", and the 2 genes coding for the two surface glycoproteins derived from the wild type The differences in shut-off temperatures were not expressed as differences in reduced virulence. The data presented in the table correlates the ca and ts markers and gene composition to the level of attenuation as measured by the response in ferrets. In all instances, where a vaccine strain has the above characteristics, it was found to be acceptably attenuated in doubly seronegative human volunteers.

AA-CR31 Clone No.	Genotype	Phenotype*	Ferret Response	Amplified**	Ferret Response
2	6 genes (ca)	39^{0}-ca	Attenuation	$10^{8.0}$	Attenuation
3	5 genes (ca) M gene (wt)	39^{0}-ca	Attenuation (dose dependent)	$10^{7.0}$ $10^{8.0}$	Reversion and mild disease
10	5 genes (ca) NS gene (wt)	38^{0}-ca	Attenuation	$10^{8.3}$	Attenuation
20	6 genes (ca)	38^{0}-ca	Attenuation	$10^{5.0}$	Attenuation
WT-A/Alaska/6/77		40^{0}	Virulent	$10^{8.3}$	Virulent
A/AA/6/60-H2N2		38^{0}-ca	Attenuation		Attenuation

*Phenotype - ts(shut-off temperature) - ca - (cold adapted) - wt - (wild type)
**Virus recovered from ferrets was amplified by passage in eggs and new sets of ferrets were infected

Influenza, Viral Genetics and the Future

996 INFLUENZA VIRAL GENETICS AND THE FUTURE, Sir Charles Stuart-Harris, Department of Virology, University of Sheffield Medical School, Beech Hill Roak, Sheffield S10 2RX England

The history of experimental genetic research on the influenza viruses began when Burnet and Lind (1) demonstrated genetic recombination, or as it is known, reassortment of the individual characters of two influenza viruses cultivated simultaneously in the same medium. Henle and Liu (2) demonstrated multiplicity reactivation of partially inactivated influenza virus thus completing the analogy with bacteriophage genetics (3). The basis of reassortment was first shown by Duesberg (4) and Pons and Hirst (5) to be the segmental structure of influenza virus RNA. Reassortment has been studied by numerous workers. Recombination of well-growing laboratory virus with a newly-isolated virus containing genes for desirable surface antigens is the basis of seed virus for inactivated vaccines. Experimental studies of the relationship between individual genes and biological properties of whole virus are dependent upon recombination techniques and determine progress with live attenuated viruses for vaccine.

The epidemiological importance of antigenic variation has long been accepted but new immunological and chemical techniques are throwing new light on the sources of the variant viruses recovered from human epidemics. Relationships demonstrated between human and animal viruses and pursued by polypeptide analysis (6) suggest that animal viruses may theoretically contribute unfamiliar surface antigens to human viruses and thus give rise to future pandemic viruses. Will theory be confirmed by practical experience of this possibility in the future and if so, can gene research lead to anticipation of the viruses of future epidemics?

The great importance of efforts to solve the problem of anticipating the antigenic changes of the viruses of future epidemics and also to obtain genetically-stable viruses for future live vaccines is underlined by the present stalemate in efforts at prevention. Future genetic research may well provide solutions to these major problems.

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Antigenic Shift and Antigenic Drift

997 HUMAN ADAPTATION TO INFLUENZA VIRAL EVOLUTION, Edwin D. Kilbourne, Department of Microbiology, Mount Sinai School of Medicine of the City of New York, New York, NY 10029 The unstable epidemiological relationship of man and influenza A viruses suggests that human genetic and physiological adaptation to infection has been minimal, probably reflecting the rarity of selective lethal disease before the end of the reproductive period. The disease affects all people, at all ages and of all ethnic groups. Fifty years ago the first isolation of a mammalian influenza virus marked the beginning of Phase I of human cerebral adaptation to the influenza viruses as the virus was studied and described. Soon, thereafter, Phase II, characterized by intervention through the use of inactivated vaccines, began with little perceptible impact on the total epidemiological picture. Quite recently we have entered a third phase of adaptation which has the potential for greatly influencing the epidemiology of influenza. In this phase, live virus vaccines containing induced mutations and laboratory produced reassortant viruses will be used. As living influenza A viruses released into the community, these viruses represent additions to the total gene pool available to wild type viruses in nature. The implications of this prospect as well as alternative stratagems for the control of influenza will be discussed.

The Viral RNA Replication and Pathogenesis

998 SEQUENCE VARIATION FOUND AT THE 3' ENDS OF NEURAMINIDASE GENE SEGMENTS FROM 25 DIFFERENT INFLUENZA STRAINS, J. Blok, Australian National University, Canberra.

It has been shown by immunological techniques that the influenza neuraminidase (NA) undergoes two kinds of antigenic variation; that of drift which are small changes within a subtype, and that of shift which results in the 9 NA subtypes (1) characterized so far.

The antigenic variation which is found correlates well with the sequence data obtained from the 3' ends of the viral RNA segment 6 (NA gene) using the dideoxy sequencing method (2). The sequence variation found in 9 N1 and 7 N2 strains ranges from 1-20% and 3-16% change in the nucleotide sequences which result in 0-30% and 3-28% difference in the predicted amino acids, respectively. These sequences represent about 15% of the total sequence and they show that antigenic drift is the result of point mutations in the RNA.

At least one strain from each of the 9 subtypes was sequenced in order to compare the antigenic subtypes at the molecular level. The nucleotide sequence following the first ATG found at the 5' end of the cDNA (≡ mRNA) is highly conserved for 20 bases. The predicted protein sequence at the N-terminus is conserved for the first 6 amino acids in all subtypes and for the next 6 in most subtypes. After this initial region of conservation both the nucleotide and predicted protein sequences show remarkably little similarity among the NA subtypes. There are no features such as Cysteines or potential glycosylation sites which are conserved in the first 100 amino acids of the predicted protein sequence from the 9 NA subtypes.

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999 EVOLUTION IN THE HAEMAGGLUTININ GENE OF INFLUENZA VIRUSES OF THE HONG KONG SUBTYPE, Merilyn J. Sleigh and Gerald W. Both, C.S.I.R.O. Molecular and Cellular Biology Unit, P.O. Box 184, North Ryde, N.S.W., 2113, Australia.

Within a subtype, influenza virus strains undergo continuous progressive changes in the antigenic character of their surface proteins, particularly the haemagglutinin. This phenomenon of antigenic drift has been shown to be due to mutations occurring within the viral gene coding for haemagglutinin, with the greatest concentration of changes occurring within the region coding for HA1, the major subunit of the protein, and the section which appears to contain all of the antibody-binding activity.

We have determined the nucleotide sequences of the HA1 coding regions of haemagglutinin genes from seven Hong Kong influenza field isolates (A/NT/60/68, A/Eng/878/69, A/Qu/7/70, A/Eng/42/72, A/Mem/102/72, A/PC/1/73 and A/Bangkok/1/79), as well as HA2 coding sequences for strains A/NT/60/68, A/Mem/102/72 and A/Bangkok/1/79. By comparing these sequences, and those published for the strains A/Aichi/2/68 and A/Vic/3/75, we have found that the rate of appearance of mutations in the gene, or of amino acid changes in the HA1 protein, is approximately constant over the period 1968-1979. The changes are concentrated in particular regions of HA1, with very few changes in HA2. Analysis of the progressive appearance of both silent and expressed nucleotide changes within the haemagglutinin gene of these strains suggests that evolution occurring early in subtype development may have followed multiple pathways, with one of these giving rise to most of the later strains in the Hong Kong subtype.

1000 SPONTANEOUS MUTATIONS AND THEIR LOCATION IN THE GENOME OF INFLUENZA A VIRUSES (H3N2). Juan Ortín, Rafael Nájera*, Cecilio López*, Mercedes Dávila and Esteban Domingo. Centro de Biología Molecular (C.S.I.C.-U.A.M.). Universidad Autónoma. Canto Blanco. Madrid-34 and *Centro Nacional de Microbiología, Virología e Inmunología Sanitarias, Majadahonda. Madrid. SPAIN.

The genetic variability of Influenza A viruses (H3N2) has been studied by comparing T1 oligonucleotide fingerprints of isolated viral RNA segments. The analysis of viruses isolated between 1968 and 1977 revealed sequence changes affecting 0,3-10,7% of the RNA positions of the genes studied, depending on the pair of viruses considered. A lower degree of heterogeneity was observed among coetaneous viral isolates. No statistically significant differences were detected in the distribution of mutations among the viral genes studied (HA NP,NA and M). The mutations within HA gene were located in HA1 two times more frequently than in HA2. Attempts to study the variability of Influenza A viruses *in vitro* are now in progress.

1001 SPECIFIC PROTECTION AGAINST LETHAL INFLUENZA VIRUS INFECTION IN MICE BY COOPERATION OF INTERFERON WITH THE HOST GENE *Mx*, Otto Haller, Heinz Arnheiter and Jean Lindenmann, Institute for Immunology and Virology, University of Zürich, CH-8028 Zürich, Switzerland.

Adult mice bearing the allele *Mx* exhibit a high degree of specific resistance to orthomyxoviruses. This resistance can be abrogated by treating the animals with anti-interferon (anti-IFN-beta) serum (Haller et al., J. Exp. Med. 149, 601, 1979). Studies *in vitro* have shown that IFN is particularly efficient in inhibiting influenza virus replication in cells bearing *Mx*. In contrast, IFN action against several unrelated viruses is not influenced by *Mx* (Haller et al., Nature 283, 660, 1980). The virus resistant phenotype is first expressed a few days after birth. This has enabled us to directly demonstrate the protective role of IFN *in vivo*. Treatment of newborn mice with exogenous mouse IFN-beta protected *Mx* carriers against lethal influenza virus challenge at doses of IFN too small to prevent death of non-*Mx* carriers. This differential protection by IFN was not seen with other challenge viruses such as EMC or VSV. We have further investigated the step at which the replication cycle of influenza viruses is arrested in IFN treated *Mx* bearing cells. Virus replication was blocked at an early stage following normal attachment and penetration but before viral protein synthesis. A mutant influenza virus not restricted by *Mx* is presently under investigation.

1002 BIOLOGIC AND GENETIC CHARACTERIZATION OF AN INFLUENZA A VIRUS ASSOCIATED WITH EPIZOOTIC PNEUMONIA IN SEALS, V.S. Hinshaw, W.J. Bean, and R.G. Webster, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

For the last two winters (1979-80) a large number of harbor seals (Phoca vitulina) have died of primary viral pneumonia on Cape Cod peninsula USA. An influenza A virus antigenically related to A/FPV/Dutch/27 (HavlNeq1) was isolated from the lungs and brains of the diseased seals. Infectivity studies of the field isolates in various mammalian and avian hosts suggested that the host range of this virus was more typical of mammalian, rather than avian, influenza A viruses. The virus produced no disease symptoms in experimentally inoculated animals. Antigenic analyses indicated that the hemagglutinin and neuraminidase of the seal isolates were closely related to those of recent viruses from birds. Comparison of the RNA of the seal virus with a large number of avian and mammalian strains showed that all genes of the seal isolate were closely related to corresponding RNAs of various avian strains. This virus, A/seal/Mass/1/80, provides the first evidence suggesting that an influenza strain deriving all its genes from one or more avian influenza viruses can be associated with severe disease in a mammalian population.

1003 GENETIC VARIATION AMONG INFLUENZA VIRUSES, James S. Robertson and Elaine Robertson, University of Cambridge, Department of Pathology, Division of Virology, Cambridge, UK.

The many different strains of type A influenza virus have been classified according to their surface antigens, the hemagglutinin and the neuraminidase. Little is known however concerning the genetic diversity of the remainder of the viral genes. This is currently under investigation by preparing dsDNA copies of individual genome segments from a variety of influenza virus strains and comparing restriction endonuclease digests. Additional analyses are being performed by direct nucleotide sequence analysis and the results will be presented.

1004 CLONING AND STRUCTURAL ANALYSIS OF THE GENOMIC RNAS OF INFLUENZA STRAINS A/USSR (H1N1) AND A/RI (H2N2), Ian W. Cummings, Patrick J. Concannon, Dean D. Gilbert and Winston A. Salser, Molecular Biology Institute, University of California, Los Angeles, CA 90024
Comparison of the genomic RNAs of influenza A viruses will be important to the understanding of the many interesting aspects of influenza virus biology. We have synthesized and cloned large double stranded DNA copies of the influenza A/USSR (H1N1) strain viral RNAs. Clones bearing nearly full length copies of the H1 hemagglutinin, N1 neuraminidase, nucleoprotein (NP) and one of the RNA dependent RNA polymerase (P) genes have been thoroughly characterized by RNA blot hybridizations, restriction enzyme mapping, melting point determinations and DNA sequencing. We are now in the process of obtaining complete DNA sequences of the major antigen genes to compare to those of other strains. We are also analyzing complementary DNA clones of the A/RI (H2N2) minus strand RNAs.

1005 SYNTHESIS OF INFLUENZA HEMAGGLUTININ IN CULTURED MONKEY KIDNEY CELLS FOLLOWING INFECTION WITH A SV40-HEMAGGLUTININ RECOMBINANT GENOME, Michael M. Sveda, Robert M. Chanock and Ching-Juh Lai, LID, NIAID National Institutes of Health, Bethesda, MD 20205
In order to express influenza viral gene sequences in mammalian cells, an SV40 vector has been used for construction of SV40-influenza viral DNA recombinant molecules. Cloned complete DNA sequences coding for the hemagglutinin of influenza A virus [A/Udorn/72(H3N2)] were inserted into the late region of a viable deletion mutant of SV40 (M. Konig and C.-J. Lai, Virology 96, 277-280, 1979). The recombinant DNA molecule was cloned and propagated in the presence of an SV40 ts mutant at a nonpermissive temperature. Infection of African green monkey kidney cells with the hybrid virus produced a protein similar to the hemagglutinin of influenza virus as detected by immunoprecipitation and polyacrylamide gel electrophoresis. Further characterization of the recombinant genome and the hemagglutinin will be discussed.

1006 BIOCHEMICAL CHARACTERIZATION OF INFLUENZA VIRUS-RESISTANT MDBK CELL VARIANT, Max Arens, M. Vijaya Lakshmi, Donna Crecelius, Carl M. Deom and Irene T. Schulze, Department of Microbiology, St. Louis University School of Medicine, St. Louis, MO 63104

We have established an influenza virus-resistant cell line which was obtained from cultures of MDBK cells that were initially persistently infected with the WSN strain of influenza A and subsequently lost the ability to produce virus. These variant cells are now almost completely refractory to influenza virus infection but retain the ability to support the growth of several other viruses including VSV, Sindbis virus, adenovirus and herpes simplex virus. All evidence indicates that this resistant cell variant was in fact derived from the completely permissive MDBK line. In view of these properties, we have termed these cells MDBK/IV to denote both their origin and their resistance to influenza virus.

We have taken several approaches to investigate the biochemical block that these cells have established to specifically prevent the replication of influenza virus. To this end, we are investigating (1) influenza virus binding and biochemical properties of the plasma membrane, (2) the production of influenza virus-specific positive and negative strand RNA, (3) the ability of the cells to accurately and efficiently translate, influenza virus specific mRNA, and (4) the possibility that virus-specific information is integrated into the cell genome.

Our experiments showed that MDBK/IV cells bound 80% less influenza virus than did the parental line and that this difference could not fully account for the lack of virus production. We have also detected the synthesis of virus-specific polyadenylated positive strand RNA in these cells and are currently characterizing this RNA and investigating its ability to be translated in vivo and in vitro. (Supported by NIAID grants AI 10097 and AI 14590).

1007 SEQUENCES OF INFLUENZA VIRUS A/PR/8/34 GENES, Stan Fields, Greg Winter, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K. and George G. Brownlee, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, U.K.

As part of a project to sequence the genome of the influenza strain A/PR/8/34 we have so far undertaken the sequences of the genes encoding the haemagglutinin (see Brownlee et al., this symposium), neuraminidase, matrix protein and non-structural proteins. Our approach is to prepare double-stranded cDNA corresponding to all virion RNA segments, cut this material with restriction enzymes and clone into derivatives of bacteriophage M13 for sequence analysis using dideoxynucleotide chain terminators. Final overlaps are then obtained by primed sequencing on virion RNA using restriction fragment primers prepared from suitable M13 clones.

The neuraminidase gene is 1413 nucleotides long, and codes for a protein containing 454 amino acids and five glycosylation sites. Comparison of the hydrophobic regions within the protein and the location of the glycosylation sites suggest that the neuraminidase, unlike the viral haemagglutinin, is oriented with its N-terminus buried in the viral membrane. The gene encoding the matrix protein is 1027 nucleotides long, of which only approximately 75% codes for this protein. There is a second long reading frame which partly overlaps that of the matrix protein which we suggest encodes an undiscovered polypeptide of about 11,000 daltons. The gene encoding the non-structural proteins is 890 nucleotides long and contains two reading frames which partly overlap. A comparison with the homologous gene from A/FPV/Rostock/34 and A/Udorn/72 (sequenced by others) confirms a high degree of sequence conservation and demonstrates that the NS2 protein is conserved at the expense of NS1.

1008 POST-TRANSLATIONAL MODIFICATION OF INFLUENZA VIRUS-INDUCED PROTEINS DURING PRODUCTIVE AND ABORTIVE INFECTIONS. M. Schrom, T. Chambers, and L. Caliguiri, Albany Medical College, Albany, NY 12208.

The replication of influenza virus is abortive in HeLa cells and the virus yield from these cells is less than 1% of that from permissive cell lines such as MDBK cells. We have previously suggested that there is a host cell defect in the final stages of virus maturation which results in a block in the release of influenza virus from HeLa cells and the accumulation of elongated budding virus particles on the plasma membrane. In the present study we have examined the synthesis and transport of viral ribonucleoprotein complexes in infected HeLa and MDBK cells and found these complexes to be synthesized normally. As detected by quantitative hybridization similar amounts of viral mRNA are synthesized in productively and abortively infected cells. The synthesis and post-translational modification of virus-specific proteins were also examined. In permissive MDBK cells infected with influenza Ao/WSN (HON1) at a multiplicity of 50 PFU/cell, the NP (nucleocapsid protein) and NS_1 (non-structural) polypeptides were detected by 4 hr after infection. In the presence of $^{32}PO_4$, increasing amounts of label were seen associated with NP and NS_1 with increasing time after infection. In similarly infected HeLa cells, NP and NS_1 were observed but were not labeled with $^{32}PO_4$. The NP polypeptide in virus released from MDBK cells was also phosphorylated; however, the small quantity of virus released from HeLa cells contained non-phosphorylated NP. This suggests that the phosphorylation of NP is important for efficient assembly of influenza virus, but not required for infectivity. However, since the NP protein is the major structural component of viral RNPs, the lack of phosphorylation of this protein may alter its charge and thus alter its association with the viral matrix protein (M), resulting in particles of aberrant morphology.

1009 MAPPING OF THE DEFECTIVE INTERFERING INFLUENZA VIRAL RNAs, N. Sivasubramanian, Silvia Hu* and Debi P. Nayak, Dept. of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024, *City of Hope Medical Center, 1500 East Duarte Rd., Duarte, CA 91010

The structure and the sequence of defective interfering (DI) viral RNAs of influenza virus L clone have been studied. Earlier studies have shown that the three DI RNAs (L1, L2 and L3) of L clone are unique in being derived from viral polymerase gene(s) via internal deletion. Two approaches were used to map these DI RNAs. 1) The cloned DNA of viral polymerase genes (vRNA segments 1, 2, 3) were hybridized with the ^{32}P-labelled specific DI RNA segments of L clone isolated from polyacrylamide agarose gels and subsequently digested with either nuclease S1 or ribonuclease A and T_1. It was found that the DI L2 and L3 RNAs were derived from internal deletion of P1 gene. Further analysis using end labelled DI RNA probes as well as ^{32}P-labelled cloned DNA segments are now in progress for specific determination of deletion. 2) Heteroduplex mapping was also done to further elucidate the structure of the DI RNAs.

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1010 EXPRESSION OF THE INFLUENZA HEMAGGLUTININ IN MONKEY AND MOUSE CELLS UTILIZING PAPOVAVIRAL VECTORS, J.R. Hartman, D.P. Nayak and G.C. Fareed, Department of Microbiology and Immunology and the Molecular Biology Institute, University of California at Los Angeles, Los Angeles, California 90024.

Deleted SV40 and polyoma viral genomes that contain the entire early gene region, the origin of replication and late leader sequences are being used to clone in monkey and mouse cells the hemagglutinin (HA) gene of influenza strain A/WSN. The HA gene - 1775 base pairs long - was purified by cloning cDNA made from viral RNA in pBR322 (Davis, Hiti and Nayak, Gene 10, 205-218, 1980). The cloned gene was further modified by treatment with nuclease Bal 31 to remove the G,C tails used for its cloning and some of the untranslated sequences, and recloned after addition of Bam H1 restriction endonuclease linkers. DNA sequencing indicated that the HA gene in different recombinants could be either expressed intact or as a fused protein from the VP2 locus of SV40 or the VP3 locus of polyoma. In both cases, expression would be under the late control elements of the vectors and the recombinants could complement in a lytic infection either deletions or ts early mutant helper viral genomes. Replication and expression of different recombinants in the monkey and mouse cell systems will be discussed.

Antigenic Variation and Prophylaxis

1011 ANTIGENIC CARBOHYDRATE DETERMINANTS ON INFLUENZA HA, Lorena E. Brown, Colin W. Ward, David O. White and David C. Jackson, Melbourne University, Parkville 3052 Vic., Aust.

Carbohydrate determinants present on the oligosaccharide side chains attached to the HA_1 of chicken egg-grown A/Memphis/72(H3), A/Japan/57(H2), A/Bellamy/42(HO) and A/shearwater/E.Aust/72(Hav6) viruses are antigenically similar, perhaps identical. Despite the differences in number, composition and location of side chains on different subtypes, anti-viral IgG raised against one of these strains reacts with the HA_1 from each heterologous subtype to the same extent and with the same overall affinity of interaction. Furthermore, adsorption of the anti-viral IgG with one of the heterologous viruses completely removes all reactivity against the other heterologous viruses. No additional antigenic carbohydrate determinants were detected on HA_1 that were not present on carbohydrate from uninfected host cells since adsorption of anti-viral IgG with host antigen removed all activity against heterologous viruses. Not all of the oligosaccharide side chains contribute to the antigenic properties of the carbohydrate on HA. Of the six side chains on the HA_1 of A/Memphis/72(H3) and X-31(H3) only those attached at residues 8 and 22 are antigenic. In A/Japan/57(H2) HA_1 only one of the four side chains is antigenic and it is also located at the N-terminus of the molecule at position 11. The single oligosaccharide unit at position 154 of HA_2 is antigenic in all three strains and the determinant(s) involved is similar to that on the antigenic side chains of HA_1. All antigenic oligosaccharide side chains are of the N-acetyllactosamine type but their compositions are not identical. Four or five N-acetylglucosamine residues and one or two fucose residues are always present but the number of mannose and galactose units is variable.

1012 DIFFERENT CELL SURFACE RECEPTOR DETERMINANTS OF ANTIGENICALLY SIMILAR INFLUENZA VIRUS HEMAGGLUTININS. S.M. Carroll, H.H. Higa and J.C. Paulson, Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

The antigenically similar RI/5$^+$ and RI/5$^-$ substrains of influenza virus (H2N2) isolated by Choppin and Tamm (J. Exp. Med. (1960) 119, 895) exhibit different properties in their interactions with erythrocytes. Once adsorbed to red cells the + substrain remains bound while the - substrain is able to elute. To determine if these properties were due to functional differences in the viral hemagglutinins which bind to cell surface sialyloligosaccharides, or in the viral neuraminidases, which hydrolyze sialyloligosaccharides, each virus was examined for its ability to interact with erythrocytes enzymatically modified (J.Biol.Chem. (1979) 254, 2120) to carry only sialyloligosaccharides with the NeuAcα2,3Gal, NeuAcα2,6Gal or the NeuAcα2,6GalNAc linkages. The results indicate that the different elution properties of the - and + viruses are due to their hemagglutinins which recognize different cell surface sialyloligosaccharide sequences as receptor determinants. The neuraminidases of both viruses preferentially cleave sialic acids contained in the NeuAcα2,3Gal sequence and very slowly hydrolyze the NeuAcα2,6Gal linkage. Thus, the - substrain rapidly elutes from red cells since its receptor determinant, NeuAcα2,3Gal, can be destroyed by the neuraminidase, while the + substrain cannot elute since its preferred receptor determinant, NeuAcα2,6Gal is resistant to hydrolysis. These results further suggest that the detailed specificities of the hemagglutinins and neuraminidases play a major role in the molecular basis for the influenza virus receptor gradient.
(Supported by NIH grants AI-16165, GM-27904 and Nat. Res. Award 07104.)

1013 TOPOLOGICAL MAPPING OF ANTIGENIC SITES ON THE PR8 VIRUS HEMAGGLUTININ USING MONOCLONAL ANTIBODIES. Michael D. Lubeck and Walter Gerhard, The Wistar Institute, Philadelphia, PA 19104

Using anti-HA monoclonal antibodies and PR8 virus antigenic mutants we have previously constructed an antigenic map of the PR8 virus hemagglutinin (HA). Two predominantly "strain-specific" antigenic sites (designated Sa and Sb) and two predominantly "cross-reactive" antigenic sites (designated Ca and Cb) were operationally defined in this analysis. In the present study, the topological relationship among these antigenic sites has been studied by competitive binding assays. The data indicate that the two "strain-specific" sites are topologically distinct from the Cb antigenic site. Various degrees of overlap were observed between the Sa and Sb, the Sb and Ca, and the Ca and Cb antigenic sites.

1014 IMMUNE RESPONSES TO INFLUENZA VIRUS IN GUINEA PIGS, MICE AND HAMSTERS, J. Stein-Streilein and M.F. Lipscomb, Department of Pathology, UTHSCD, Dallas, Texas. 75235

Human Influenza PR/8/34 was inoculated into the lungs of guinea pigs, mice and hamsters. Where as .026 hemagglutinating units (HAU) of the virus was lethal to mice, 256 HAU were tolerated by guinea pigs and hamsters. Guinea pigs remained clinically free of disease. Hamsters became ill but survived. Morphologic studies of the lungs of hamsters and mice with influenza infection demonstrated similarities to histologic changes seen in lungs from humans with influenza pneumonia. The kinetics of both cell mediated (thymus derived lymphocyte, T-cell) and antibody (Bursa derived lymphocytes, B-cell) responses were studied in a variety of lymphoid tissue. T cell responses (^{3}HTdR incorporation) were widely dessiminated throughout the animals' lymphoid tissue as early as one week. Antibody forming cell responses (Jerne plaque) appeared restricted to the draining lymph nodes (hilar). In the hamster, the quality of the T-cell response varied with the strain of hamster used in the experiment. The serum antibody (hemagglutination inhibition) and antibody forming cell response in the guinea pig and hamster appeared to be dependent on the viability of the virus inoculum. Inactivated virus was incapable of stimulating an antibody forming cell response, was markedly less capable than live virus for stimulating serum antibody, but was adequate for stimulating T cells to proliferate. These studies demonstrate that the hamster and the guinea pig are useful for the study of immune responses to antigens inoculated into the lungs as well as providing a model for the study of influenza pneumonia.
(Supported in part by USPHS Grant HL23870)

1015 ANALYSIS OF TRYPTIC GLYCOPEPTIDES OF THE HEMAGGLUTININ GLYCOPROTEIN OF H_1N_1 INFLUENZA VIRUSES BY REVERSE PHASE HPLC, Sukla Basak and Richard W. Compans, University of Alabama in Birmingham, Birmingham, Alabama 35294

We have analysed the glycosylated tryptic peptides of the hemagglutinin (HA) glycoprotein of several H_1N_1 strains of influenza virus in order to investigate the extent of variation in the glycosylation sites among closely related virus strains isolated in the USA, Japan and Brazil during the year 1977-78. ^{3}H-glucosamine labeled HA glycoproteins were isolated from purified virions by polyacrylamide gel electrophoresis, reduced and alkylated, and subsequently subjected to complete trypsin digestion. Glycosylated tryptic fragments were separated by reverse phase high performance liquid chromatography using a linear gradient of 0 to 30 parts of n-propanol in 0.1% phosphoric acid. Eight distinct glycosylated tryptic peptides were obtained for the HA glycoprotein of the A/USSR/90/77 strain. Analysis of other strains showed that A/California/10/78 does not differ significantly from the A/USSR strain, whereas one additional new peak was detected for the A/Lackland/AFB/3/78 and two new peaks were detected for the A/Fukushima/103/78 strain. Moreover, when compared with A/USSR virus, significant alterations were found in most of the tryptic peptides of the HA of A/Lackland/7/78, A/Brazil/11/78 and A/Arizona/14/78 strains. These results demonstrate that differences exist in the HA glycosylation sites among closely related strains of the H_1N_1 subtype of influenza virus. Further analysis of the separated tryptic fragments may provide further information on the role of glycosylation in the antigenic variation of influenza virus.

1016 HETEROGENIETY OF INFLUENZA VIRUSES ISOLATED FROM THE HOUSTON COMMUNITY OVER A SIX YEAR PERIOD, Howard R. Six, W. Paul Glezen and Cynthia Griffis, Baylor College of Medicine, Houston, TX 77030

Epidemic influenza has occurred in Houston during the last six winters. Analysis of isolates from each year indicate that major viruses were A/Port Chalmers (H3N2) in 1975, A/Victoria (H3N2) in 1976, B/Hong Kong in 1977, A/Texas (H3N2) in 1978, A/Brazil (H1N1) in 1979, and B/Singapore in 1980. During 3 of the type A epidemics, a relatively low frequency of type B viruses were isolated. Type A viruses were isolated during both type B epidemics. Late in the H3N2 epidemic of 1978 125 influenza H1N1 viruses were isolated. A serological characterization of H3N2 viruses isolated during 1975-1978 indicated that both A/Victoria and A/Texas variants were present and that the percentage of A/Texas isolates during 3 consecutive winters was 0.4%, 30% and 70%, respectively. Comparison of hemagglutinin proteins of 18 A/Victoria strains isolated during this period by competition radioimmunoprecipitation (RIP) assays showed that 50% differed antigenically from the prototype strain. Most of the H1N1 strains isolated during 1978 were antigenically similar to A/USSR but 7% were identified as A/Brazil the dominant virus of 1979. However, several other H1N1 variants were also isolated. Analysis of 230 H1N1 strains with seven monoclonal antibodies raised to A/USSR indicated that 29% of the 1978 and 13% of the 1979 isolates differed antigenically from the dominant strain. These data indicate that influenza viruses isolated from the same community within the same epidemic are heterogeneous. However, most of the antigenic differences are relatively small and detectable only with monoclonal antibodies or by competition RIP assays.

1017 THE THREE-DIMENSIONAL STRUCTURE OF THE A/HONG KONG/68 HAEMAGGLUTININ AND ITS IMPLICATIONS FOR ANTIGENIC VARIATION, Ian A. Wilson, John J. Skehel and Don C. Wiley, Harvard University, Cambridge, MA 02138

The structure of the bromelain-released haemagglutinin glycoprotein of influenza virus has been determined at 3Å resolution. The trimer consists of two structurally distinct regions. The region closest to the membrane is more fibrous with a central triple-stranded coiled-coil arrangement of α-helices, 76Å in length, and a five-stranded antiparallel β-sheet with residues from both HA_1 and HA_2. A globular region of only HA_1 residues sits on top of the fibrous region and consists mainly of antiparallel-β-structure with a central eight-stranded "swiss-roll" type β-sheet. A concave pocket on top of this globular region containing several invariant residues forms a possible receptor site for binding to host cells. Each subunit has an unusual loop-like topology beginning at the membrane extending 135Å distally to form the two structural regions and folding back to enter the membrane with the interesting possibility that the molecule may be anchored at both ends during the folding process by the signal peptide and hydrophobic tail embedded in the membrane. The structure has been analysed with reference to the available primary sequence data on natural and laboratory selected antigenic variants. Four antigenic sites have been identified on the surface of each subunit. At least one amino acid substitution has been observed in each site of the haemagglutinins of recent strains and thus appears to be required for the production of a new epidemic.

1018 GENETIC AND BIOCHEMICAL ANALYSIS OF THE INFLUENZA A/ANN ARBOR/6/60 COLD-ADAPTED MUTANT, Nancy J. Cox, Ingo Konnecke, Alan P. Kendal and Hunein F. Maassab*, Centers for Disease Control, Atlanta, Georgia 30333; *University of Michigan, Ann Arbor, Michigan 48104.

We are attempting to fully characterize the cold-adapted and temperature-sensitive mutant of influenza A/Ann Arbor/6/60 which is a parent donor strain for the production by recombination of live,attenuated vaccine strains. Genetic analysis of the A/Ann Arbor/6/60 mutant virus by its recombination with a series of wild-type or mutant viruses indicates that the cold-adaptation and temperature-sensitivity properties are multigenic, requiring the presence of two or more genes from the mutant parent. Using a variety of techniques, including oligonucleotide mapping, peptide mapping and comparative polyacrylamide gel electrophoresis of double stranded RNA hybrids, we have detected lesions in all eight RNA segments of the A/Ann Arbor/6/60 mutant. In order to determine what effect these lesions might have on virus replication, examination of virus-specific polypeptide synthesis at restrictive temperatures has been undertaken. The presence of multiple RNA lesions in the attenuated mutant A/Ann Arbor/6/60 and the reproducible transfer of the non-glycoprotein genes to candidate recombinant vaccine strains supports the use of this method of preparing live attenuated vaccines since such recombinants are likely to be stable and to have predictable levels of attenuation.

1019 Ir GENE CONTROL OF THE CYTOLYTIC T LYMPHOCYTE RESPONSE TO INFLUENZA VIRUS. Carol S. Reiss, Martin E. Dorf, Baruj Benacerraf, and Steven J. Burakoff. Sidney Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

We have investigated the immune response (Ir) gene control of the cytolytic T lymphocyte (CTL) response to influenza virus in mice. Although most strains respond equally to H-2K or H-2D antigens in association with influenza virus, we observed that one strain (C57BL/6, K^bD^b) and its F_1 progeny (b x k, b x d, b x a, b x q) failed to mount a detectable response to one end, $H\text{-}2K^b$ plus virus. This defect was detected in responses to challenges by either type A or type B viruses. In contrast, mice congenic with the defective C57BL/6 strain which share $H\text{-}2K^b$, but possess either $H\text{-}2D^d$ or $H\text{-}2D^q$, were found to lyse infected targets which were compatible at $H\text{-}2K^b$. Using a panel of H-2 recombinant congenic strains, we were able to demonstrate that the gene(s) which controlled this immune response map in or adjacent to the H-2D region. This contrasts with other Ir genes, such as those for antibody production, which map to the H-2I region. Thus, in order to respond to $H\text{-}2K^b$ plus virus, $H\text{-}2D^d$ or $H\text{-}2D^q$ genes had to be in the cis configuration.

1020 AN INFLUENZA IMMUNOSOME: ITS STRUCTURE AND ANTIGENIC PROPERTIES. A MODEL FOR A NEW TYPE OF VACCINE, Lise Thibodeau and Armand Boudreault, Institut Armand-Frappier, Univ. du Québec, Laval, Québec, Canada H7N 4Z3

The current influenza virus vaccines made of inactivated particles often induce undesirable local and general pyrogenic reactions particularly among youngsters. There is a need for a vaccine that would not contain the substances responsible for these side effects; this can be done by eliminating viral lipids, nucleic acids and proteins other than the antibody-inducing surface glycoproteins. We have made a selective extraction of these proteins and constructed an immunosome, which is exclusively made of hemagglutinin and neuraminidase attached onto the membrane of a preformed liposome. The phospholipids are solubilized with the detergent β-d-octylglucoside and the liposomes are formed by the injection method. The hemagglutinins are extracted from purified influenza viruses with Triton N-101. The solubilized glycoproteins are purified by centrifugation through a discontinuous sucrose gradient; the non dialysable Triton is exchanged during the centrifugation for β-d-octylglucoside that is incorporated into the gradient. When the hemagglutinin subunits, free of detergent, are mixed with preformed liposomes, the incorporation on the surface membrane is extremely low. This difficulty can be overcome by mixing a protein-detergent complex with preformed liposomes, the membranes of which had been "fluidified" in the presence of minute amount of detergent. A decreasing detergent gradient dialysis allows the exchange of the proteins from detergent to the phospholipid bilayer. This technique recreates a structure that is almost undistinguishable from the virus particle. This immunosome is highly immunogenic and possesses an hemagglutinating activity much greater than the viral subunits.

1021 ANTIGENIC CHARACTERIZATION OF INFLUENZA A VIRUS NUCLEOPROTEIN, Kathleen L. Van Wyke*, William J. Bean, Jr., and Robert G. Webster, St. Jude Children's Research Hospital, Memphis, TN 38101 (*present address: LID, NIAID, National Institutes of Health, Bethesda, MD 20205)

Antigenic variation in the nucleoprotein (NP) of influenza A viruses was studied using monoclonal antibodies. Enzyme-linked immunosorbent assays (ELISA) of these antibodies indicated that the WSN/33 (HON1) influenza virus NP possesses at least five distinct antigenic determinants, and that variation in some of these determinants has occurred in other influenza A strains. Competitive binding ELISA assays showed that the five determinants comprise three non-overlapping antigenic domains on the NP molecule. Monoclonal antibodies to two of the NP domains inhibited viral RNA synthesis in vitro, suggesting that these specific regions of the NP molecule are topographically or functionally involved in RNA transcription.

1022 ANALYSIS OF THE SPIKE GLYCOPROTEIN OF INFLUENZA C VIRUS. Herbert Meier-Ewert[+], Arno Nagele[+], Georg Herrler[++], Sukla Basak[++] and Richard W. Compans[++], [+]Techn. Univ. Munich/ Germany and [++]Univ. of Alabama, Birmingham, Al. 35294

We have recently reported that in contrast to influenza virus types A and B, influenza C virions possess only a single glycoprotein gene product (gp I). This precursor glycoprotein requires a specific posttranslational proteolytic cleavage to yield two glycoprotein subunits, gp 65 and gp 30, linked together by disulfide bonds. Posttranslational cleavage of viral glycoproteins as a prerequisite for full biological activity is a characteristic which is shared by all myxo- and paramyxoviruses.

In order to study isolated viral glycoproteins, purified virions were fractionated by selective solubilisation with nonionic detergents. We describe here some biochemical properties of isolated influenza C glycoproteins, such as sugar components and determination of amino acid swquence at the N-terminus for the two subunits of the viral surface projections. In addition, we report about some morphological aspects of the organisation of the spikes on the viral envelope, as well as their organisation after the release from the viral membrane by protease treatment or upon spontaneous disruption.

1023 HOW DOES NEUTRALIZED INFLUENZA VIRUS BECOME NON-INFECTIOUS? Nigel J. Dimmock and Robert D. Possee, Department of Biological Sciences, University of Warwick, Coventry, England.

Contrary to popular belief, neutralized influenza virus is taken up by cells to the same extent and at the same rate as infectious virus preparations. Neutralized virus is uncoated within the cell and viral ribonucleoproteins transported to their usual locations but no virus proteins are synthesized. Evidence bearing on the viral function that is inhibited by antibody will be presented.