OPTIONS FOR THE CONTROL OF INFLUENZA

Alan P. Kendal and Peter A. Patriarca, Organizers

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Molecular Approaches to Understanding the Biology of Influenza Viruses

1823 CHARACTERIZATION OF CHICKEN/PENN/84, W.J. Bean, Y. Kawaoka, V.S. Hinshaw and R.G. Webster, St. Jude Children's Research Hospital, Memphis, TN 38101.

In April 1983, an influenza virus of low virulence appeared in chickens in Pennsylvania. In October 1983, a virulent virus appeared and caused high mortality in poultry. The agent was identified as an influenza virus of the H5N2 serotype. Comparison of the genome RNAs of chick/Penn with other influenza viruses indicated that all of its genes were closely related to those of various other isolates from wild birds and domestic turkeys. Analysis of the RNAs of the viruses isolated in April and October by RNA-RNA hybridization and oligonucleotide mapping indicated that these strains were very closely related and RNA sequence analysis of their hemagglutinin genes showed only seven nucleotide changes. Polyacrylamide gel analysis of the avirulent isolates demonstrated the presence of low molecular weight RNA bands indicative of defective interfering particles that were not detected in the virulent isolates. Experimental infection of chickens with mixtures of the avirulent and virulent strains demonstrated that the avirulent virus interferes with the pathogenicity of the virulent virus.

The results suggest that the original avirulent virus was probably derived from influenza viruses from wild birds and that the virulent strain was derived from the avirulent strain by selective adaptation rather than by recombination or the introduction of a new virus into the population. These viruses should provide a useful model system for the study of the adaptation of an influenza virus to a new host, the acquisition of virulence and the mechanism of interference.

1824 EXPRESSION OF INFLUENZA POLYMERASE PPOTEINS, Thomas M. Chambers, David R Londo, and Debi P. Nayak, UCLA School of Medicine, Los Angeles, CA 90024

From our study of the defective-interfering (DI) particles of influenza virus, it has become evident that both the generation of DI particles and the interference caused by DI particles are processes involved with the mechanism of influenza virus replication. The agents of replication, the three influenza polymerase proteins, are poorly understood regarding their tertiary structure; active sites; and their interactions with each other, with the ribonucleoprotein template, and with the nascent RNA strand. To study polymerase structure-function relationships we have generated specific antibodies directed against bacterially expressed proteins containing a portion of the E. coli trp LE' peptide fused to several different regions of each polymerase protein. Also, we have expressed individual polymerase proteins in eukaryotic cells using an SV40 vector system, allowing us to study the effect of alterations in primary structure on polymerase function.

1825 INTERACTION OF M PROTEIN AND RNP OF INFLUENZA VIRUS IN VITRO. Ghendon, Y., Mikheeva, A., Melnikov, S. Moscow Research Institute for Viral Preparations, USSR.

When the M protein isolated from purified orthomyxovirus virions was added to the transcription in vitro system containing RNP of orthomyxoviruses inhibition of transcription was observed that reached 90%. The inhibition was based on the formation of the M protein: RNP complex and this formation is salt-dependent but not NP-40-dependent, indicating electrostatic but not hydrophobic character of links formed. An orthomyxovirus mutant was detected having a ts mutation of the M protein with an altered character of links of the M protein with RNP. Analysis of products formed in in vitro system in the presence of the M protein revealed impairment in the transcription of all RNA segments, transcription of high molecular weight RNA segments being inhibited more significantly than that of low molecular weight RNA segments. Calculation of stoichiometric ratios of the M protein: RNP complex suggests that a link of the M protein with vRNA is more probable.

1826 VARIATION IN INFLUENZA VIRUS RECEPTOR SPECIFICITY, James Paulson, Gary Rogers, Herman Higa, Jun-Ichiro Murayama, Tom Pritchett and Gloria Sze. Department of Biological Chemistry, UCLA School of Medicine, Los Angeles CA 90024. Influenza viruses bind sialyloligosaccharide groups of glycoproteins and glycolipids as cell surface receptors, and in general recognize a receptor determinate that encompasses more than the terminal sialic acid residue. For example, of influenza isolates containing the H3 hemagglutinin, human isolates preferentially bind receptors containing the SAα2,6Gal linkage, while avian and equine isolates bind the SAα2,3Gal linkage. These differences are sufficiently different to allow selection of a receptor variant of the contrasting receptor specificity from either a human or avian isolate, by applying suitable selective pressure to favor propagation of the variant. Closer examination of cloned SAα2,6Gal specific viruses derived from several different (H3) parent viruses has revealed additional differences in receptor binding properties. These include differences in the recognition of sialyloligosaccharides with N-acetyl- and N-glycolyl-neuraminic acids as the terminal sialic acid, in the sensitivity to inhibition of plaque formation by equine α_2-macroglobulin, in the agglutination of erythrocytes of different species, and in the ability to propagate in chicken embryos with retention of receptor specificity. These observations indicate that influenza virus receptor binding properties and the biological consequences of receptor specificity cannot be entirely predicted simply by preferential binding to SAα2,6Gal or SAα2,3Gal linkages.

1827 CHANGES IN THE HEMAGGLUTININ OF INFLUENZA A (H1N1) VIRUS DURING EGG ADAPTATION, James S.Robertson, Janet S.Bootman, Robert Newman, Rod S.Daniels, Clayton W.Naeve, Robert G.Webster and Geoffrey C.Schild, National Institute for Biological Standards and Control, London NW3 6RB UK and St Jude Childrens Research Hospital, Memphis, Tn. USA

Schild et al (1983, 1984) have demonstrated that antigenic variants of influenza A (H1N1) and B viruses are selected during egg-adaptation of clinical isolates. Sequence analyses of the HA genes of these viruses before and after egg-adaptation reveal specific amino acid substitutions associated with the ability of the viruses to grow in the allantoic cavity of embryonated eggs. Many of these substitutions modify the reactivity of the viruses in haemagglutination-inhibition tests using either monoclonal or polyclonal antibodies. These findings have strong implications on the use of egg-adapted virus for epidemiological studies, virus research and possibly for vaccine production.

Schild,G.C., Oxford,J.S., de Jong.J.C. and Webster,R.G. (1983). Nature 303, 706-709.
Schild,G.C., Oxford,J.S. and Webster,R.G. (1984). In 'The Molecular Virology and Epidemiology of Influenza (C.H.Stuart-Harris and C.W.Potter, eds) pp 163-171, Academic Press, London

1828 Physiological characterization of ts mutants defective in HA and M genes, A.Sugiura and M. Ueda, The Institute of Public Health, Tokyo Japan. We characterized the physiological defect in ts mutants of WSN strain of influenza virus having mutation in HA or M gene. MDBK cells infected with either of two HA mutants, ts-61S and ts-134, synthesized at the nonpermissive temperature HA polypeptide identifiable by immunoprecipitation and immunofluorescence. The polypeptide was, however, prevented from maturing into fully glycoslated HA and migrating from Golgi area to the cell surface. The mutants were releaved from the block by temperature shift-down. The mutation responsible for the above physiological block also conferred the unusual heat-lability to the virus produced at the permissive temperature. Cells infected with each of three M mutants, ts-51, ts-62, and ts-9/12, also synthezied at the nonpermissive temperature all virus-specific polypeptides including M_1 protein. Immunofluorescent staining with antisera specific to M_1 protein showed that M_1 protein was confined to the perinuclear region in mutant-infected cells in contrast to wild type virus-infected cells in which M_1 was present over the entire cytoplasm. HA was expressed on the surface of both mutant- and wild type virus-infected cells. The HA on the mutant-infected cells was unusually susceptible to antibody-mediated redistribution, suggesting the increased lateral mobility of HA on the plasma membrane. The finding is compatible with the absence of M_1 protein, thought to anchor the HA expressed on the cell surface, underneath the plasma membrane.

Open Topics on Influenza

1829 INFLUENZA VIRUSES AS LYMPHOCYTE MITOGENS, E. Margot Anders, Anthony A. Scalzo, Sally L. Paine, Pantelis Poumbourios, David C. Jackson and David O. White. Department of Microbiology, University of Melbourne, Parkville, Vic. 3052, Australia.

Many influenza A viruses behave as B lymphocyte mitogens in vitro. B cell proliferation occurs independently of T cell help and its magnitude varies with the HA subtype of the virus, the order for BALB/c mice being H2 > H6 ≥ H3, with H1 viruses being only poorly mitogenic. At least two different mechanisms of mitogenesis appear to be involved. H3 viruses are equally mitogenic for all strains of mice tested. In contrast, a mitogenic response to H2 and H6 viruses is restricted to strains of mice that express the Class II MHC glycoprotein I-E (haplotypes a, d, k, p, r). Purified H2 hemagglutinin (HA) is also mitogenic and follows the same pattern of genetic restriction as H2 virus. These and other data suggest that mitogenesis by H2 (and probably H6) viruses is initiated by the direct binding of viral HA to I-E molecules on the B cell surface.

The extent to which the response to influenza virus in vivo is influenced by the mitogenic capacity of the virus is under study. The properties of immunogenicity, adjuvant activity and polyclonal activation of Ig secretion are being compared for viruses of high and low mitogenic capacity, and for H2 and H6 viruses in $I\text{-}E^{+}$ (responder) and $I\text{-}E^{-}$ (low responder) strains of mice.

1830 THE CHARACTERISTICS OF NEURAMINIDASE ASSOCIATED WITH A BIFUNCTIONAL PROTEIN OF AN INFLUENZA VIRUS, D. Jit S. Arora and L. Fouchard Gabriel, Virology Research Center, Institut Armand-Frappier, University of Quebec, L-D-R, Laval, H7N 4Z3.

We first reported the presence of two antigenically distinct neuraminidases (NA and NAH) in a wild influenza strain A/Aichi/2/68 (H3N2). The enzyme NA possesses only the NA activity whereas the enzyme NAH also possesses the hemagglutinin activity. The present communication is concerned with the comparative characteristics of NA and NAH. The optimum pH at which their activity was maximal, were 5.5 for NA and 4.5 for NAH when N-acetyl neuraminyllactose (NAN-LAC) was used as substrate. Results of heat stability experiments showed that incubation at 50 C for 30 min reduced the activity of NAH by 91% compared to 31% loss for NA. The activity of NAH enzyme decreased considerably in the presence of merthiolate and copper ions compared to that of NA. The two enzymes differed in their Km values for NAN-LAC, -2,3-methoxyphenyl-N-acetyl neuraminic acid. However, their Ki values for several inhibitors were not different.

These results showed that though these two enzymes catalyzed the same reaction yet they differed in a number of fundamental properties.

1831 Excess Pneumonia and Influenza Associated Hospitalization During Influenza A Epidemics in the U.S., 1970-1978
William H. Barker, Department of Preventive Medicine, University of Rochester, Rochester, N.Y. 14642

While excess mortality due to epidemics has been measured in a variety of ways from published vital statistics, excess hospitalization, which probably represents the largest cost to the health care system, has not been measured and reported. In this study, excess rates of pneumonia and influenza (P&I) associated hospitalization during influenza A epidemics which occurred between 1970-1978 were computed utilizing unpublished data from the National Hospital Discharge Survey. Statistically significant excesses occurred at rates of 35, 93 and 370 per 100,000 persons per epidemic for age groups 15-44, 45-64 and 65 + years of age. There was no evidence of a persisting excess or a compensatory decline in P&I hospitalization during post-epidemic months. An average excess of 172,000 hospitalizations per epidemic at cost in excess of $300 million was computed. The study quantifies a major impact upon health and health services, much of which may be preventable (1), and illustrates an important use of unpublished data contained in the NHDS.

1. Barker WH, Mullooly JP. Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths. JAMA 1980;244:2547-49.

1832 CHANGES IN BIOLOGICAL CHARACTERISTICS OCCURRING ON ADAPTATION OF DIFFERENT INFLUENZA A VIRUSES TO INCREASED VIRULENCE IN MICE, Earl G. Brown, Influenza Section, Viral Diagnostic Services Division, Laboratory Centre for Disease Control, Tunney's Pasture, Ottawa, Ontario K1A 0L2 Canada

Virulent mutants of influenza virus derived from avirulent parents are needed to analyze the molecular-genetic basis of virulence in influenza A viruses. Virulent influenza A mutants were derived by serial passage in mice of 10 prototype viruses representing the 3 influenza A subtypes. Comparison of the virulence, infectivity, growth kinetics in vivo and in vitro as well as antigenicity were made between pairs of mouse adapted and parental viruses. Low virulence viruses tended to increase in virulence on passage in mice although they did this to different extents and after different numbers of passages. All viruses that increased in virulence also grew better in mouse lungs although the correlation between these two factors was not proportional. Mouse adapted viruses were seen to possess various combinations of the other altered characteristics with no single or group of characteristics being consistently observed. The data suggested that more than one characteristic and thus more than one gene can contribute to pathogenicity. Influenza viruses of increased virulence have been produced and biologically characterized; they will be used to analyze the molecular-genetic basis of the biological phenomenon of pathogenicity.

1833 MECHANISMS OF NEUTRALIZATION OF INFLUENZA VIRUS BY MONOMERIC AND POLYMERIC ANTIBODIES, Howard P. Taylor and Nigel J. Dimmock, University of Warwick, Coventry, U.K.

Using purified secretory(s) IgA and IgM we have compared mechanisms of neutralization with those mediated by IgA monomers (prepared from the sIgA by differential reduction) and IgG. With BHK cells held at 4^{o}, sIgA and IgM prevented attachment of neutralized virus while neither monomeric IgA or IgG had any effect on this process or on the subsequent stages of infection by which virion RNA accumulates in nuclei. At 25^{o} and 37^{o}C, sIgA and IgM permitted attachment of approximately half the neutralized virus but it was not internalized. Clearly the mechanism of neutralization depends on the character of the antibody used: sIgA may act by steric hindrance (with attachment or penetration, depending on temperature) whereas IgA and IgG neutralize infectivity at a stage subsequent to accumulation of the virus genome in the nucleus.

1834 INFLUENZA B REINFECTION, Arthur L. Frank, Cheryl M. Porter and Larry H. Taber, Baylor College of Medicine, Houston, TX 77030

Persons in the Houston Family Study have been observed for influenza B infection since 1975. Virus cultures were obtained during each illness and serum was collected before and after the influenza season. Infections were identified by virus isolation &/or $\geq$ 2 $\log_2$ increase in serum microneutralization titer. Influenza B outbreaks occurred in 1977 (HK-like), 1980 (Singapore), 1982 (Singapore) and 1984 (USSR). Thirty-six children observed from birth and followed through all 4 outbreaks had at least 32 infections (1984 serology pending); 81% were infected at least once and 3 reinfections occurred. For the entire population there were 696 person-seasons at risk where prior infection history was known. For 1980 and 1982, persons with no infection during the previous epidemic had infection rates of 25% and 26%, respectively, while those infected during the previous outbreak had rates of 16% and 4%. For 1982, those persons without infection for 2 prior outbreaks were infected at a rate of 36% while those with known infection 2 epidemics previously had a rate of 9%. Reinfections were identified only in children; 7 were school-age and 4 were ages 3-5. Two of 11 reinfections were associated with an isolate, 8 were inapparent or associated with mild illness, and 3 with fever &/or influenza. In 1984, 22 (7.5%) of 292 persons had isolates and 6 (all in children) were reinfections, 4 associated with fever &/or influenza. Four children had had isolates during their previous infections. These data indicate a definite but incomplete protective effect of prior natural infection, apparently influenced by antigenic changes.

1835 INFLUENZA IN TAIWAN, 1977-1984, Hsin-Chih. Wang and Hsiu-Yeuh. Wei, Veterans General Hospital, VACRS, Taipei, Taiwan, ROC.

Studies on epidemiology of influenza in Taiwan in the period October 1977-July 1984 has been carried out, swab specimens were collected from 1226 patients with acute respiratory infections and were subjected to standard isolation and identification techniques in PMK (or MDCK) tissue culture.

A total of 370 strains of influenza viruses were isolated (30.2%) including 10 A/USSR/90/77(H1N1), 12 A/Brazil/11/81(H1N1), 10 A/England/333/80 (H1N1), 1 A/Chile/1/83(H1N1), 20 A/Texas/1/77(H3N2), 15 A/Bangkok/1/79(H3N2), 66 A/Philippine/2/82(H3N2), 88 B/Hong Kong/5/72, 145 B/Singapore/222/79, and 3 B/Illinois/1/79.

The outbreak of influenza A/USSR/90/77(H1N1) and influenza B/HK/5/72 occurred between October 1977 and August 1979. During the summer of 1979-1980, most reported sporadic cases and outbreaks in Taiwan were attributed to influenza A/Bangkok/1/79(H3N2) viruses. Influenza B/Singapore/333/79 viruses were isolated from sporadic cases and outbreaks occurred in this area beginning in June 1980, and by the end of July 1984, isolates had also reported. The first confirmed epidemic of influenza A/Philippine/2/82(H3N2) in Taiwan was reported in mid-January 1983 and school absenteeism increased.

1836 INTERACTION OF INFLUENZA VIRUS HEMAGGLUTININ WITH MEMBRANE ERYTHROCYTES AND ISOLATED PLASMA MEMBRANES OF CELLS, M.K.Indulen, V.I. Bubovich, G.M.Ryazantseva and V.A.Kalnina, August Kirchenstein Institute of Microbiology, Latvian SSR Acad.Sci., Kleisti, 226067 Riga, Latvian SSR, USSR

We have studied the hemolytic activity of a number of strains of influenza virus type A (H1N1, H2N2, H3N3, H7N1 and H7N7) and type B, both, sensitive and resistant to rimantadine. All the viral strains of serotypes H1N1, H2N2 and H3N3 possess the maximum of hemolytic activity within pH 5.0-5.25. The resistant variants of these strains are more stable to pH changes towards alkalification. Serotypes H7N1 and H7N7 are active within pH 5.25-5.5, while B-type influenza viruses - within 5.5-6.25. The use of isolated plasma membranes of CEF and MDCK cells demonstrated, that upon a neutral medium pH the virus (infectious and with ^{3}H-uridine-labelled RNA) adsorbed on membranes, may fuse with the membrane, that results in the ribonucleoprotein core becoming sensitive to RNase. Rimantadine in concentrations 0.25 mM and 0.5 mM does not prevent membrane fusion.

The work discusses the possible link between the ability of influenza viruses to lyze erythrocytes upon an acid medium pH and the mechanism of virus penetration and uncoating in sensitive cells.

1837 MOLECULAR CHANGES IN H5N2 AVIAN INFLUENZA VIRUS ASSOCIATED WITH ACQUISITION OF VIRULENCE, Y. Kawoaka, K.L. Deshpande, C.W. Naeve and R.G. Webster, St. Jude Children's Research Hospital, Memphis, TN 38101

The highly virulent influenza virus that appeared in chickens in Pennsylvania in 1983 illustrates the potential threat of influenza to animals, as well as humans. The virus first isolated from chickens in April 1983 caused low mortality but in October 1983 the virus became highly virulent and caused up to 80% mortality. Further outbreaks occurred in turkeys in Virginia through June 1984. The causation agent has been characterized as an H5N2 influenza A virus. The virus has apparently been eradicated from domestic poultry by depopulation of over 17 million birds at a cost of approximately $61 million.

The molecular changes that occurred in the virus with the acquisition of virulence are under investigation. The comparative sequence and cleavability of the hemagglutinin molecule have been determined, the sequence of the neuraminidase gene is in progress and the contribution of the other genes to the acquisition of virulence are under investigation.

1838 Evaluation of a Cold-Recombinant (CR) Influenza B Vaccine. W.A. Keitel,* T.R. Cate, R.B. Couch. Baylor College of Medicine, Houston, Texas.

Live attenuated virus vaccines are being explored as an alternative to parenteral inactivated vaccines for prophylaxis against influenza. This report describes the early evaluation of a CR influenza B vaccine bearing the surface antigens of B/Hong Kong/73, or CR7.

25 healthy young adult volunteers received approximately 6.8 $\log_{10}TCID_{50}$, or an estimated 10 median human infectious doses of CR7 by nose drops. Two volunteers developed mild, afebrile upper respiratory illness following inoculation. Infection rates varied according to preinoculation serum neutralizing antibody (NtAb) titers, as shown below:

Pre NtAb	No. of Volunteers	No. with Virus Shedding	≥4x NtAb Rise	No. (%) Infected
<2	8	6	5	8 (100)
3-12	12	4	6	8 (66)
≥ 16	5	0	0	0

Three to five months after inoculation 10 CR7 recipients and 7 unvaccinated volunteers were challenged with wild-type (wt) B/Houston/76. All CR7 recipients resisted wt challenge. Three of 7 unvaccinated volunteers developed respiratory illness; five shed virus; and 4 had a significant NtAb rise following wt challenge.

These studies confirm that CR7 is attenuated, immunogenic, genetically stable and capable of protecting against wt challenge. Protection was strongly correlated with increasing levels of prechallenge serum NtAb.

1839 EPIZOOTIOLOGY OF INFLUENZA, Charles J. Kelleher, David A. Halvorson and Dennis A. Senne, University of Minnesota, St. Paul, MN 55108 and Nat. Vet. Services Laboratories, Ames, IA 50010.

A five-year study (1980-1984), involving sentinel ducks and sentinel turkeys that intermingled with wild waterfowl and surveillance of domestic turkey flocks, has been conducted to investigate the interspecies transmission of influenza A virus. Isolation-reared, seven-week-old mallard ducks were placed in pens on selected marshes during mid-May or June to November each of five years and cloacal samples were collected biweekly in 1980 and weekly thereafter. Seven to nine-week-old domestic turkeys were placed within the pens during 1982-1984 in an attempt to identify highly diffusible influenza A virus isolates. Sentinel turkey tracheal and cloacal samples were collected weekly and blood samples were collected biweekly. Commercial turkey flocks were also monitored for virologic/serologic evidence of influenza.

Results indicate that the onset of infection among sentinel ducks was similar each year (mid-July to August). In addition, outbreaks of influenza among domestic turkey flocks also appeared to be seasonal with the onset of infection occurring six to eight weeks after the initial detection of infection among sentinel ducks during four of five years. Despite an often high infection rate among sentinel ducks, sentinel turkeys had only three detectable infections in three years, indicating that only certain influenza isolates circulating among waterfowl at any given time are capable of infecting turkeys.

1840 INTERFERENCE WITH A CONFORMATIONAL CHANGE IN THE HEMAGGLUTININ MOLECULE OF INFLUENZA VIRUS BY ANTIBODIES AS A POSSIBLE NEUTRALIZATION MECHANISM Hiroshi Kida, Sayaka Yoden, Mikinori Kuwabara and Ryo Yanagawa, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

Monoclonal antibodies to each of four antigenic areas on the hemagglutinin(HA) molecule of A/seal/Mass/1/80(H7N7) neutralized infectivity of the virus and inhibited virus-induced hemolysis whereas those of groups III and IV failed to inhibit hemagglutination of the virus. At pH 5.9, which is optimal for hemolysis, hemagglutination of intact virus or HA rosettes was not inhibited with any of the antibodies, indicating that a conformational change in the HA molecule occurred. On the other hand, hemagglutination-inhibition was observed when the antigens were incubated with the antibodies at pH 7.0 and then the pH was shifted to 5.9, suggesting that antibodies prohibit the conformational change. Fab fragments of groups III and IV antibodies could no longer neutralized viral infectivity nor inhibit hemolysis. However, infectivity of the virus bound with Fab fragments of these antibodies was effectively neutralized by addition of anti-Fab fragment antibodies.

Using a spin-labeling method combined with electron spin resonance (ESR), a low pH-induced conformational change in the HA molecule of seal virus was detected. Changes in the ESR spectrum were observed at pH value corresponding to the optimal for the hemolysis activity of the virus, confirming that a conformational transition in the HA molecule occurred at this pH. The changes in the spectrum were suppressed by antibody-binding.

Based on these results, we propose a possible mechanism of neutralization of influenza virus by antibodies to the HA, in addition to the generally accepted mechanism of blocking attachment to host cell receptors. This involves interference with a low pH-induced conformational change in the HA molecule by bivalent binding of antibodies, which results in inhibition of the fusion step in the viral replication process.

1841 **HEMAGGLUTININ TRANSPORT MUTANTS**, Hans-Dieter Klenk, Wolfgang Garten, Wilhelm Schuy, Kazumichi Kuroda, Hiroshi Naruse and Christoph Scholtissek, Institut für Virologie, Justus-Liebig-Universität D-6300 Giessen, Germany

Mutants of influenza virus A/FPV/Rostock/34 (H7N1) have been obtained by chemical mutagenesis which show ts defects in the transport of the hemagglutinin from the rough ER to the plasma membrane. The amino acid sequence of the hemagglutinin has been derived from nucleotide sequence analyses. By comparison with wild type virus, point mutations were detected which appear to be responsible for the transport defects.

1842 **CONSTRUCTION OF AN INVERTEBRATE EXPRESSION VECTOR FOR THE FPV HEMAGGLUTININ GENE**, Kazumichi Kuroda, Wilhelm Schuy, Wolfgang Garten and Hans-Dieter Klenk, Institut für Virologie, Justus-Liebig-Universität, D-6300 Giessen, Germany

The c-DNA of the FPV hemagglutinin gene was cloned into the PstI site of plasmid pUC8 using the G/C homopolymer tailing technique. The G/C regions were eliminated by Bal31 exonuclease digestion and new cleavage sites (Hind III) were introduced at both ends of the hemagglutinin gene with synthetic oligonucleotides. The deleted c-DNA will be transferred into the baculovirus expression vector pAc 380 to obtain expression in insect cells.

1843 STRUCTURAL PROPERTIES OF THE INFLUENZA C GLYCOPROTEIN SPIKE, Herbert Meier-Ewert and Arno Nagele, Depart. of Med. Microbiology, Techn. University Munich, Biedersteiner Str. 29, 8 Munich 40, F.R.G.

In contrast to influenza A and B viruses, influenza C posseses only a single type of surface spike, gp 88, which similarly to the hemagglutinin is made up of two subunits, gp 30 and gp 65. Exposure of purified influenza C virions to 1% mercaptoethanol releases the larger subunit gp 65 into the supernatant, thus furnishing direct chemical evidence about the orientation of the glycoprotein molecule. This confirms the sequence data suggesting that gp 30 is inserted into the viral membrane, while gp 65 is the distal portion of the spike. In addition, we showed by in situ crosslinking experiments with purified virions, using formaldehyde as a chemical cross-linker that a trimer of the monomeric form of gp 88 was the most abundant multimer to be found in protein gels. Analysis of the sugar/protein ratio of the trimer, together with the reversion of the crosslinking upon heating in aqueous solution revealed the homopolymeric nature of the protein band in a second dimension.
This study demonstrates the trimeric structure of the influenza C glycoprotein by direct chemical means.

1844 STUDIES OF INFLUENZA VIRUS GLYCOPROTEIN MUTANTS GENERATED IN VITRO N. Sivasubramanian and D.P. Nayak. Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024.

Influenza virus surface antigens viz., hemagglutinin (HA) and neuraminidase (NA) are both integral membrane proteins and are incorporated into virions. SV40 vectors were used to express cDNA clones of these viral glycoproteins in mammalian cells. HA and NA expressed from cloned cDNAs were glycosylated and transported to the cell surface of monkey kidney cells and also possessed biological activities of native viral antigens. We wanted to elucidate the mechanisms of intracellular processing and subsequent transport of these viral proteins to the cell surface. For this purpose, alterations like internal deletions, point mutations, insertions were made on the cloned cDNAs of the viral glycoproteins through conventional recombinant DNA techniques. The alterations were made on the various hydrophobic and hydrophilic regions of these proteins and their involvement in the synthesis, glycosylation, and transport to the cell surface were studied. The phenotypes of these mutant proteins will be discussed.

1845 PROTECTIVE EFFECT OF MURAMYL DIPEPTIDE ON INFLUENZA VIRUS INFECTION IN MICE, Philip R. Wyde, Howard R. Six and Robert B. Couch, Influenza Research Center, Houston, TX 77030

The effects of two known macrophage activators, muramyl dipeptide (MDP) and its n-butyl derivative, murabutide (mura), on severe influenzal disease was evaluated in mice. Untreated mice or mice inoculated intranasally (i.n.) or intraperitoneally (i.p.) with multiple doses of MDP were challenged with 2- to 6-median lethal doses (LD_{50}) of mouse adapted influenza A/HK/68 virus i.n. During a fourteen day interval the cumulative mortality in each group of animals was recorded and compared. In two experiments, no significant differences in deaths were observed between mice inoculated i.n. with 100 µg of MDP on days -4, -1, 1 and 4 and control mice. In contrast in both of these experiments, marginal protection was observed in mice inoculated i.p. with this dosage and schedule ($p \leq 0.2$). In experiment 3, MDP concentrations were increased to 1000 µg per dose; again no protection from lethal disease was observed in mice given multiple doses i.n. However, this same dose and schedule of inoculation given i.p. afforded total protection to the mice. In a separate experiment 250 micrograms of the mura derivative given in multiple inoculations i.p. also afforded good protection. These experiments indicate that MDP and its derivatives may be useful in lessening the severity of influenza pneumonia, but dose and route of inoculation are important.

1846 INFLUENZA VIRUS ADSORPTIVE MUTANTS SELECTED BY MONOCLONAL ANTIBODIES, J.W.YEWDELL, A.CATON, and W.GERHARD. WISTAR INSTITUTE, PHILA. PA.

To investigate the effect of a polyclonal antibody response on viral evolution, A/PR/8/34 was grown in vitro in the presence of increasing concentrations of a mixture of neutralizing anti-HA monoclonal antibodies. Three variants selected under these conditions were characterized in detail. Although the variants were virtually indistinguishable antigenically from parental virus as assessed by the binding of 103 monoclonal anti-HA antibodies in an indirect RIA, most antibodies demonstrated decreased titres to the variants in HI tests. Hemagglutination mediated by the variants was found to be less sensitive to alkaline pH or neuraminidase treatment of erythrocytes. Based on these findings we concluded that the variants represent adsorptive mutants with increased avidity for host cell receptors. Dideoxy sequencing revealed each of the variants to have a single unique amino acid alteration in its HA1 subunit. Two of the changes are in proximity to the proposed receptor binding site, and could conceivably directly alter receptor binding, while a third is located near the trimer interface, and would most likely increase receptor binding by altering monomer-monomer interactions.

1847 SINGLE-DOSE PHARMACOKINETICS AND NASAL MUCUS PENETRATION OF AMANTADINE HCl AND RIMANTADINE HCl IN ADULTS, Frederick G. Hayden and Howard E. Hoffman, University of Virginia, Charlottesville, VA 22908 and Stine-Haskell Laboratory, Newark, DE 19711

The pharmacokinetics of amantadine HCl (A) and rimantadine HCl (R) were compared in a two period crossover study involving six young (19-35 years) and six elderly adults (60-70 years). Subjects ingested single 200 mg oral doses after an overnight fast, and serial plasma (0-96 hr), nasal mucus (0-8 hr), and urine (0-24 hr) samples were tested for A or R concentrations (C) by HPLC assay.

Although no significant age-related differences were noted, R differed significantly from A in mean (SD) plasma Cmax [0.25(0.05) vs 0.72(0.21) ug/ml], plasma elimination t1/2 [34.6(11.2) vs 14.5 (5.2) hr], and % dose excreted in 24 hr urine [0.6(0.8) vs 45.7(15.7)]. Urinary excretion of R metabolites averaged 18% of the administered dose. Despite significantly lower C plasma, the mean (SD) C nasal mucus of R were similar to A, and the ratios of C nasal mucus/C plasma were higher after R than A (*$p \leq 0.05$, A vs R):

Drug (n)	Hr post dose	C plasma (ug/ml)	C mucus (ug/gm)	Ratio
A (12)	4	0.51(0.13)*	0.28(0.26)	0.59(0.61)*
	8	0.45(0.17)*	0.39(0.34)	0.95(0.86)*
R (12)	4	0.24(0.05)	0.26(0.25)	1.05(0.92)
	8	0.20(0.03)	0.34(0.21)	1.75(1.10)

The high ratio of C nasal mucus/C plasma suggested active transport of R into respiratory secretions. These findings may in part explain the prophylactic and therapeutic effectiveness of R in influenza A virus infections at dosages that have lower toxicity than those of A.

1889 CLONED MURINE CYTOTOXIC T LYMPHOCYTES PROMOTE RECOVERY OF MICE INFECTED WITH INFLUENZA VIRUS, Aron E. Lukacher, Vivian L. Braciale, and Thomas J. Braciale, Washington University School of Medicine, St. Louis, MO 63110

The capacity of cytotoxic T lymphocytes (CTL) to specifically lyse virus-infected syngeneic cells in vitro represents a potential recovery mechanism from viral infection in vivo. In order to examine the in vivo effector function of CTL, we have studied the ability of adoptively transferred influenza virus-specific cloned CTL lines to promote recovery of mice from lethal influenza pneumonia. We have found that CTL clones with in vitro specificity for a given type A influenza virus subtype mediate recovery of mice infected only by that virus subtype. In contrast, CTL clones which exhibit cross-reactive recognition of all type A viruses in culture likewise promote recovery in a cross-reactive fashion. Recovery correlates with reduction of lung virus titers and bsence of gross pulmonary lesions. Importantly, when mice are simultaneously infected with two different virus subtypes, a subtype-specific CTL clone eliminates pulmonary virus of the recognized subtype but not of the other virus subtype and therefore fails to prevent death. This finding suggests that an antigen-specific effector function is utilized by CTL in vivo (e.g., direct lysis of infected cells) rather than through a nonspecific process (e.g., lymphokine release). [This work was supported by USPHS grant AI-15608 and Medical Scientist Training Program Grant 5 T32 GM-07200].

Properties of Influenza Viruses — Epidemiology and Immunology

1848 THE IMMUNE RESPONSE TO INFLUENZA A VIRUS INFECTION, Gordon L. Ada, Department of Microbiology, Australian National University, Canberra, Australia. 2601.

It is convenient to divide infection by influenza virus in a suitable host into three stages. The first is the infection process whereby invading virus particles infect both susceptible (epithelial) and non permissive (macrophages) cells. The second is the influence of a variety of non-specific mechanisms in limiting the extent of replication which occurs. The third is the recovery phase which results in the clearing of virus from the infected organ. In non-immune hosts, infection is readily achieved; specific antibody is the main means of preventing infection. It is generally accepted that only antibody to certain epitopes of the hemagglutinin molecule will efficiently neutralize virus and prevent infection; there is continuing debate about the mechanism of the neutralization process. Infection of macrophages may be the process which initiates a specific immune response. They do not support productive infection but they effect antigen presentation and recruitment of lymphocytes by interleukin production. Alpha interferon is released and this has a direct anti-viral effect and modulates the activities of other cells such as NK cells. Both the classical and alternate pathways of complement are activated and contribute to lessening the extent of virus infection. Helper T cells are formed. These are cross reactive against influenza A strains and are required for IgG and IgA production, and at least in vitro for effector T cell production - cytotoxic (Tc) cells and delayed-type hypersensitivity (Td) cells. Specific antibody given to mice after infection will reduce virus levels in the lung and a variety of in vitro tests indicate possible mechanisms. However, it is thought that effector T cells with cytotoxic activity are mainly responsible for reduction of lung viral titres. Infectious virus is more effective than inactivated virus at causing Tc cell production and these cells are cross protective across A strains. Tc cells recovered from infected hosts or generated in vitro are class I antigen restricted but recently some clones of Tc cells have been shown to be class II antigen restricted. In the murine model, class II antigen restricted cells which have DTH but not Tc activity do not lower lung viral titres on transfer and contribute to the lung pathology observed. Clearance of nasal virus in humans correlates with the degree of Tc cell activity which can be generated from peripheral blood cells. The mechanisms of action of Tc cells in vivo is of considerable interest and will be discussed.

1849 IMPACT OF EPIDEMICS UPON COMMUNITIES AND FAMILIES, W. Paul Glezen, Howard R. Six, Arthur L. Frank, Larry H. Taber, Dennis M. Perrotta, and Michael Decker, Influenza Research Center at Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 and the School of Public Health, University of Texas Health Science Center at Houston.

Influenza viruses have produced epidemics of acute respiratory disease (ARD) in Houston during every year for the past ten years.[1,2] The peak of ARD hospitalizations and the peak of visits for ARD to primary care facilities have coincided with the peak of influenza virus activity for each year. The severity of the epidemics has varied depending upon the age-specific susceptibility of the population to the prevalent virus. With the cooperation of the Texas State Health Department, surveillance activities have been extended to other cities in Texas, and the data show simultaneous occurrence of influenza epidemics with the same morbidity pattern as Houston.

Currently used methods for estimating excess mortality for the United States underestimate the serious morbidity associated with influenza epidemics. Surveys of ARD hospitalizations in Houston have shown that more than 15 persons are hospitalized with ARD for each pneumonia death that occurs during epidemic periods. Analysis of underlying conditions of 8003 persons hospitalized with ARD during the influenza epidemics of 1978-81 show that only 40.6% had an underlying condition for which vaccine is currently recommended. Given vaccine efficacy of 70% we could expect prevention of only 28.4% of ARD hospitalizations during epidemics even if all "high risk" patients were immunized each year. Of 514 deaths among 8003 ARD hospitalizations, 441 (85.8%) occurred among persons with "high risk" conditions or over 65 years of age. Given immunization of all these patients we might expect prevention of 309 (62.1%) deaths.

Immunization of all high risk patients would have little effect on epidemic influenza or the perennial high morbidity among children and young adults. The average annual rate of illness caused by influenza viruses among persons followed in the Houston Family Study has been 26 per 100 persons. The rate has been highest for school children at 37 per 100 and preschool children at 32 per 100. The illness rate for their parents was about one-half the rates for the children. New approaches to prevention will be required to reduce serious morbidity associated with epidemic influenza.

1. Glezen WP (1982). Serious morbidity and mortality associated with influenza epidemics. Epidemiol Rev 4:25-44.
2. Glezen WP, Six HR, Perrotta DM, Decker M, Joseph S (1984). Epidemics and their causitive viruses - community experience, in The Molecular Virology and Epidemiology of Influenza (eds, C. Stuart-Harris and C.W. Potter) Academic Press, London, pp 17-31.

1850 EPIDEMIOLOGICAL CHARACTERISTICS OF TYPE C INFLUENZA, Morio Homma, Department of Microbiology, Kobe University School of Medicine, Japan

Outbreaks of type C influenza have rarely been documented and the virus has been isolated only occasionally. This is attributable to the subtle clinical manifestations of the disease. Thus epidemiological information on type C influenza is very limited. We attempted to determine the age distribution of the antibody to type C influenza virus in the residents of Yamagata Prefecture located in the north east of Japan(1). A total of 186 sera were collected from healthy residents in the age range of 0-80 years in 1976 and 436 sera from the other individuals in the same community in 1979. All the newborn infants had matternal HI antibody which disappeared during the first 6 months after birth. The number of the antibody positive sera became detectable from 1 year of age, then increased rapidly and reached a range of 80-90% by the age of 7-10 years. The antibody positive ratio was maintained beyond the age of 70. The age-related antibody distribution pattern with the sera of 1974 was quite similar to that obtained with the sera of 1976. These results suggested that periodic fluctuations of type C influenza epidemics, if present, occurred at intervals of not longer than 1 year. Since HI titers were maintained at higher levels, the possibility of the occurrence of reinfection was considered. Principally, the same antibody distribution pattern was obtained from the study of the residents in Iriomote Island, the southmost part of Japan, suggesting that the virus is widespread in whole Japan (2). On the basis of the above knowledges, a one-year survey of a children's residence was undertaken that housed twenty children aged 0-3 years and had 29 employees aged 24-56 years (3). During the survey period, an outbreak of type C influenza unassociated with other known respiratory infections was detected. Sevevteen of 20 children and 2 of the 9 employees who volunteered developed symptoms characterized by fever and nasal discharge. The 3 children under 3 months of age did not contract the disease. A total of 13 strains of the virus were recovered and identified. Evidence of reinfection was found serologically in a child and 2 adults and the virus was recovered from one of the latter. These findings suggest that the reinfection occurs soon after primary infection and frequently thereafter. The followup study will be reported which supports the above view.

References; (1) Homma, M. et al. Microbiol. Immunol. 26, 639, 1982. (2) Kaji, M. et al. Kurume Med. J. 30, 121, 1983. (3). J. Inf. Dis. 148, 51, 1083.

1851 MODELING INFLUENZA EPIDEMICS, Ira M. Longini, Jr., Department of Statistics and Biometry, Emory University, Uppergate House, Atlanta, GA 30322

A review is given of the mathematical and statistical models that have been used to investigate the epidemiology, prediction and control of influenza epidemics. Statistical models have been used to analyze the transmission of influenza in households when illness or serological data have been collected in community studies. Such analyses have yielded measures of the infectiousness of various types and subtypes of influenza in different community settings. Mathematical and statistical models also have been developed for studying the transmission of influenza in communities with structured populations. Stochastic simulation models have been employed to investigate the efficacy of different vaccine distribution patterns in the community population. In addition, deterministic models have been developed to find the optimal distribution of a limited supply of vaccine among various age groups in the population. These models reveal the relationship between the optimal control strategy and the nature of agent behavior, e.g., A(H2N2) vs A(H3N2), the underlying objectives of epidemic control, the age structure of the population and the quality and quantity of available vaccine.

Models also have been used to analyze influenza activity on a national and even global scale. Time series analysis of pneumonia-influenza deaths reported from cities in the U.S. has been employed by the CDC to predict influenza epidemics. These predictions are made when a predetermined number of weekly totals (or sometimes percentage of total deaths) exceed an "epidemic threshold" which is based on expected mortality from non-epidemic years. Improvements and the implications of this method are discussed. Deterministic models of the geographic spread of influenza have been used in the Soviet Union for 20 years. These models utilize the transportation network and estimated parameters from the ascending limb of the epidemic curve in the first city experiencing an epidemic to predict the geographic spread of influenza for the rest of the territory modeled. More advanced models are now being employed to predict the possible pandemic spread of influenza. These models could be instrumental in determining the best global distribution of vaccine needed to prevent or control the next pandemic strain of influenza.

The contribution of modeling to influenza epidemiology and control is given along with recommendations for future modeling work. Future applications involve models for the explanation and prediction of antigenic drift and shifts. Statistical models could prove to be useful in measuring the degree of viral interference among cocirculating types and subtypes of influenza. Optimal control models should prove to be instrumental in guiding policy decisions about the distribution of vaccines on regional, national and global levels. In addition, the possible role of the Soviet model of geographic spread as part of a world influenza surveillance system will be discussed along with its other possible applications.

1852 GLOBAL EPIDEMIOLOGY OF INFLUENZA A AND B VIRUSES. M.S. Pereira and P. Chakraverty, Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London. NW9 5HT

Although it is rarely possible to predict the impact of influenza with any certainty the surveillance system co-ordinated by the World Health Organisation at least ensures that the influenza viruses circulating in all parts of the world are detected and available for antigenic analysis and for vaccine production where this is indicated.

However, outside the infrequent appearance of a new sub-type when florid epidemics are instantly recognisable influenza is rather poorly monitored as a disease and the essential evidence of whether a new variant has epidemic potential is often not available. Various methods for improving this type of surveillance have been proposed but in only a few countries have these been implemented.

The present procedures allow some success by refinements in the techniques available for the selection of variants for vaccines but the inability to forsee antigenic changes in the viruses or to anticipate their likely effect on populations reduces their effectiveness. It has also become apparent that outside pandemic periods when similar viruses are found world-wide even neighbouring countries may have quite different influenza viruses predominating at the same time.

All these factors have important implications for mounting successful vaccination campaigns.

Control through Use of Inactivated Vaccines

1853 EXPERIENCES IN THE USE AND EFFICACY OF INACTIVATED INFLUENZA VACCINES IN NURSING HOMES. N Arden-Kater, PA Patriarca, AP Kendal, Influenza Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, GA 30333.

Influenza viruses are important causes of nosocomial infection in nursing homes and other chronic-care facilities in the U.S. (1). Outbreaks may affect up to 60% of patients, many of whom die or develop life-threatening complications. Although inactivated influenza vaccine has been recommended to reduce the incidence and severity of influenza in these settings the effectiveness of these programs has been demonstrated only recently.

In 1983, Patriarca et al retrospectively evaluated the impact of a community-wide influenza A (H3N2) epidemic on 1,476 residents of 13 nursing homes in Genesee County, Michigan (2). While the overall efficacy of influenza vaccine in preventing influenza-like illness was 32%, similar to findings in previous studies (1), the efficacy in preventing hospitalizations, pneumonia, and death was higher, ranging from 47-79%. Prospective studies conducted in two other nursing homes during the 1982-83 influenza season yielded similar results (3,4).

While the findings in these and other studies support current influenza vaccine recommendations, relatively few residents in many nursing homes are vaccinated in any given year. In a survey of 8,354 residents in 67 nursing homes in six states in 1982 and 1983, the proportion vaccinated ranged from 8-98%; homes that required consent from family members had lower rates of vaccination than homes that did not ($p<.0001$; median 57% vs. 90%, respectively). Data collected from other sources also indicate that well organized vaccination programs are more successful than others. Thus, distribution of educational materials to assist family members in making informed decisions, and development of practical guidelines to assist nursing home administrators in improving vaccine delivery, may further increase the number of nursing home residents who are immunized.

1. Patriarca PA. Influenza vaccine. Breaking the vicious cycle. In: Proc 18th Immuniz Conf, 1983. Atlanta, Centers for Disease Control, pp. 175-6.
2. Patriarca PA, Weber JA, Parker RA, et al. Efficacy of influenza vaccine in nursing homes. JAMA (in press).
3. Gross PA, Quinnan GV, Rodstein M, et al. Association of influenza immunization with reduction in mortality in an elderly population. In: Proc 24th Intersci Conf on Antimicrob Agents and Chemother, 1984. Washington, D.C., Amer Soc Microbiol, Abstract #288.
4. Betts RF, Dolin R, Treanor JJ, Roth FK, O'Brien D. Inactivated influenza vaccine reduces frequency and severity of illness in the elderly. Ibid., Abstract #289.

1854 EFFICACY OF INFLUENZA VACCINE AMONG NON-INSTITUTIONALIZED ELDERLY PERSONS William H. Barker, Department of Preventive Medicine, University of Rochester School of Medicine, Rochester, N.Y. 14642 and John P. Mullooly, Kaiser Health Services Research Center, Portland, Ore. 97215

Annual influenza vaccination of the elderly has long been recommended by the U.S.P.H.S. However, the majority of these "high risk" persons fail to receive vaccine, partly due to lack of studies of vaccine efficacy in this age group. The authors have conducted retrospective analyses of vaccine efficacy in reducing pneumonia and influenza (P&I) associated morbidity and mortality among non-institutionalized elderly persons during four epidemics caused by strains of H3N2 influenza A. The study population consists of elderly members of a large prepaid practice. Dates of excess P&I mortality, prevalent strains of influenza A, strains included in vaccine, and percent elderly vaccinated for each epidemic were as follows:

	I	II	III	IV
Excess Mortality	Dec'68-March'69	Dec'72-March'73	Jan-April'76	Dec'80-March'81
Epidemic Strains	A/Hong Kong/68	A/England/72	A/Vic/75	A Bangkok/79
Vaccine Antigen	A/Japan/62 (H2N2)	A/Hong Kong/68	A/P. Chalm/73 A/Scotland/74	A Bangkok/79
%Vaccinated ≥ 65 Y/O				
Without High Risk	16.5	12.5	18.0	15.5
With High Risk	28.4	22.9	26.7	30.5

During each epidemic vaccinated and non-vaccinated subgroups of elderly persons were comparable in mean ages, distribution and severity of underlying chronic disease.

A/Hong Kong vaccine attained a 72% (31%-100%) reduction in P&I associated hospitalization and 87% (52%-100%) vaccine reduction in mortality during the 1972-73 epidemic. A/Bangkok vaccine attained 72% (26%-89%) reduction in hospitalization and a reduced mortality rate that approached statistical significance during the 1980-81 epidemic. A/Japan vaccine and the A/Port Chalmers-A/Scotland vaccine failed to protect against hospitalization or death during the 1968-69 and 1976 epidemics, respectively. These vaccine failures appear attributable to a relatively weak immunologic relatedness between vaccine hemagglutinin antigen and hemagglutinin of the epidemic strain. (Personal communications A. Kendall and R. Couch).

In sum influenza vaccination confers substantial protection upon elderly persons when vaccine antigen is immunologically closely related to antigen of the prevalent epidemic virus.

Barker W, Mullooly J. Am J Epidemiol 1980;112:798-811 and JAMA ,1980;244:2547-9.

1856 EXPERIENCES WITH MASS VACCINATION OF YOUNG AGE GROUPS WITH INAC_ TIVATED VACCINES, Akira Oya,Department of Virology and Rickettsiology, National Institute of Health, 2-10-35,Kamiosaki, Shinagawa, Tokyo 141,Japan.

Split type of inactivated influenza vaccine has been applied extensively to school children of 5 to 15 years in Japan for these 12 years. Politically immunization of those children is mandarory though it has not been applied so strictly. Approximately 50 per cent of target population have received 2 dosis of vaccine every year. Claims for side reaction reported to the central committee has been few, being less than one per million of vaccinees though compresensive follow-up of the vaccinee has not been carried out in whole country. No definite syndrome as Guillain-Barre syndrom has not been assessed being particularly related to the vaccination. As for the efficacy of immunization, a number of report has been issued and results varied depending upon the epidemic years. However, those reports mostly concern infection of the vaccinee with influenza virus. Report is seldom which deals with the impact of immunization to the community. An assessment will be made on the effect of immunization particularly on the influenza in the community introducing some reports including that dealt with the relation between coverage rates of vaccination in children and the whole rates of absenteeism of children in schools.

1857 WHO NEEDS INFLUENZA VACCINE?,Frederick L. Ruben,Department of Medicine,Montefiore Hospital,University of Pittsburgh School of Medicine,Pittsburgh, PA 15213

Killed influenza vaccines are currently the most important available tool against epidemic influenza. For over twenty years it has been recommended that killed influenza vaccines be given annually to all persons with high-risk conditions. The high-risk conditions have been broadly defined to include persons of all ages with any chronic disease and all persons over 65 years of age. Vaccine was also advised for special populations who provide essential services. The basis for these recommendations has been the cumulative experience, primarily from pandemics, beginning with observations of Farr in 1847 and continuing through data on the Asian pandemic of 1957. The wisdom of administering influenza immunization annually, whether the virus has undergone antigenic shift or merely antigenic drift, is born out by the excess mortality observed not only during pandemics but during "mild" seasons of influenza activity as well. Despite the recommendations, vaccine has reached only a small percentage of high-risk persons, and there is no evidence that mortality from epidemic influenza is being controlled.

Studies of epidemic influenza over the past two decades suggest that a reassessment of the high-risk population is needed. Investigators from Houston[1] and Portland[2] have data from influenza epidemics on pediatric and adult hospitalizations and on mortality which provide specific rates by age and by categories of underlying conditions. Fedson[3] has documented that up to three-fourths of persons dying from pneumonia or influenza were hospitalized in the preceding year. Medical personnel, formerly considered for vaccine only in terms of being providers of essential services, are being considered as potential transmitters of influenza to high-risk patients[4]. Reports continue to demonstrate the severe impact of influenza in chronic care facilities[5,6]. From these recent data derived from interpandemic influenza, a redefinition of high-risk conditions permits the setting of priorities for targeting the available supplies of killed vaccines. Assessment of efforts to control epidemic influenza should logically follow.

1. Glezen WP, Epidemiol. Rev. 4:25-44, 1982
2. Barker WH, Mullooly JP, Arch. Intern. Med. 142:85-89, 1982
3. Fedson DS, Primary Care 4:761-799, 1977
4. Prevention and Control of Influenza, MMWR 33:253-266, 1984
5. Goodman RA, Orenstein WA, et al. JAMA 247:1451-1453, 1982
6. Influenza Outbreak, Louisiana. MMWR 33:15, 1984.

Regulation and Manipulation of the Immune Response to Influenza Virus

1858 ROLE OF ACCESSORY CELLS, T CELL DIFFERENTIATION FACTOR AND OTHER LYMPHOKINES IN HUMAN INFLUENZA A VIRUS-SPECIFIC CYTOTOXIC T CELL RESPONSES

D. Bruce Burlington, Susan C. Kiley, Jay P. Siegel, Martha A. Wells and Gerald V. Quinnan, Jr., Division of Virology, FDA, Bethesda, MD 20205

Virus-specific cytotoxic T Lymphocyte (CTL) activity occurs in humans infected with or immunized against influenza A viruses. Extensive murine and limited human data suggest that CTL responses are important in controlling virus replication and limiting illness after influenza virus challenge. To analyze the modulation of these responses by lymphokines we have examined the in vitro generation of HLA-restricted virus-specific CTL responses from resting human lymphocytes. Peripheral blood mononuclear leukocytes (PBL) were separated into monocytes (MO) and highly purified small T cells (T cells) by plastic adherence and by sequential depletion of nylon wool adherent cells, sedimentation through discontinous percoll density gradients, and depletion of Fc gamma bearing cells. PBL or MO and T cells were cultured with virus for 6 days in RPMI 1640 medium and 5% human AB serum with or without lymphokines or polyclonal lymphokine-specific neutralizing antisera. The supernatants of virus stimulated PBL cultures contained: alpha interferon (IFN)-present by 6 hr. (max. 3000 IU/ml at 2 to 6 days); gamma-IFN present by 18 hr. (max 1000 U/ml at 3 to 6 days); and interleukin-2 (IL-2) present by 24 hr. (max 1.5 U/ml at 2 to 3 days). Culture of PBL with exogenous alpha and gamma IFNs had minimal effects on CTL generation. However, neutralization of alpha IFN decreased proliferation 25% and decreased CTL responses. Neutralization of gamma IFN decreased proliferation 75% and prevented CTL responses from occurring. Addition of 10 U/ml of recombinant DNA produced IL-2 to PBL cultures increased proliferation and increased nonspecific cytolytic activity more than virus-specific CTL. When small T cells were stimulated with virus both MO and exogenous IL-2 were needed to induce proliferation. However, small T cells cultured with 3% MO and pure IL-2, even if also supplemented on day 3 with alpha and/or gamma IFN, did not induce CTL effectors. In contrast, supplementation on day 3 with 15% conditioned supernatant from Con-A stimulated PBL did induce specific CTL effectors as did co-cultivation with either virus infected large granular lymphocytes or PBL when each was separated from the responding T cells by a 0.22 micron membrane.

These experiments demonstrated that in virus stimulated cultures of unfractionated PBL, CTL effectors develop under positive regulation by alpha IFN, gamma IFN and IL-2. However, in virus stimulated cultures of small T cells supplemented with these lymphokines proliferation occurs but CTL are not induced. Differentiation of T Cells into CTL effectors requires supplementation with an additional lymphokine which can be provided by conditioned supernatants from Con-A stimulated PBL or through co-cultivation with PBL or large granular lymphocytes. The action of the additional lymphokine(s) therefore, appears to be that of a human T cell differentiation factor.

1859 SPECIFIC VH/Vk COMBINATIONS WHICH RECOGNISE RELATED EPITOPES ON THE INFLUENZA VIRUS HEMAGGLUTININ.

A.J.CATON, G.G BROWNLEE, L.M.STAUDT and W.GERHARD. UNIV. OXFORD,UK and WISTAR INSTITUTE, PHILADELPHIA, PA.

Sequence analysis of heavy and light chain mRNAs from hemagglutinin-specific hybridomas (obtained following primary and secondary immunisation of Balb/c mice with influenza virus A/PR/8/34) reveals that a group of hybridomas of related specificity express members of the same VH family in specific association with particular Vk regions derived from different Vk gene families. A detailed comparison of probable somatic mutations reveals that while the VH regions appear to accumulate replacement mutations which affect the fine specificity of these antibodies, the Vk regions undergo mutation under a strong selection to maintain germ-line amino acid sequence. These results may then indicate both positive and negative influences of somatic mutation on the clonal expansion of B cells bearing this specificity.

1860 IMMUNOGENICITY OF AN ISOLATED DETERMINANT OF THE INFLUENZA HEMAGGLUTININ.(HA) FOR THE HELPER T CELL(T_H) RESPONSE IN MICE, Charles J. Hackett, Julia L. Hurwitz, Bernhard Dietzschold, and Walter Gerhard. The Wistar Institute, Philadelphia PA 19104 . The anti-HA response of BALB/c mice immunized with whole PR8 (H1N1) influenza virus is characterized by the frequent isolation in longterm lines or T hybridomas of T_H directed to the amino acid 111-119 region of the HA1 polypeptide. All such T_H respond in vitro to a synthetic peptide of this sequence, which has been shown using shortened homologues to be the minimal or nearly-minimal determinant. All T_H specific for this site recognize all tested human isolates of subtype H1 influenza viruses from 1934-1980, but fail to respond to a mutant HA having $Glu_{115} \rightarrow Lys$. Further, these T_H can be subdivided into two fine specificity groups by their response to a Swine influenza.

Immunization of BALB/c mice with the isolated determinant covering this region (amino acids 111-120) elicited T_H which recognized whole PR8 and the other human H1 viruses, as well as the peptide. No T_H were found which recognized peptide but not whole virus. Both fine specificities of recognition on Swine virus were represented among the peptide-elicited T_H. This great similarity in recognition specificity between T_H elicited by whole virus and those from peptide immunizations suggests that the synthetic peptide of residues 111-120 contains the complete immunogenic determinant for BALB/c T_H clones responding to this part of the HA molecule.

1861 ONSET OF CELL-MEDIATED IMMUNE FUNCTION (CMI) AFTER BONE MARROW TRANSPLANT COINCIDES WITH CESSATION OF CHRONIC SHEDDING OF INFLUENZA A VIRUS (FLU A). C.J. Harrison, L.J. Jenski, T. Sketch, and M.J. Gilchrist. Children's Hospital Research Foundation, Cincinnati, Ohio.

Over nine months, 15 Flu A (H1N1) isolates with similar RNA oligonucleotide maps and titers of were obtained from a patient with SCID. He underwent unsuccessful lectin separated (LS) marrow transplant without preconditioning in August 1983 and successful LS transplant with preconditioning in November 1983. He had recurring febrile respiratory infections with pulmonary infiltrates (only two bacterial isolates) prior to transplant. Five unexplained febrile episodes occurred during the time of Flu A shedding.

CMI and leukocyte surface markers were followed. Four weeks after the second transplant, NK activity was detected against CMV, HSV, influenza B, and K562 targets, but not against targets infected with his Flu A. At seven weeks, mature T cells were demonstrated by monoclonal antibodies, and normal T and B cell numbers were detected by 16 weeks post transplant. Lymphocyte proliferation to mitogens was detected first at 9 weeks and levels comparable to normal controls were achieved at 15 weeks. NK activity to Flu A targets was not noted until 13 weeks, and CTL to Flu A were not found until 15 weeks post transplant. Flu A specific NK and CTL activity persisted at 35 weeks post transplant. Thus, cessation of viral shedding at 13 weeks coincided with the onset of Flu A specific NK and subsequent CTL activity. Clinical signs due to Flu A ceased after the first transplant though engraftment was unsuccessful until the second transplant.

1862 HUMAN MONOCLONAL ANTIBODIES TO INFLUENZA A VIRUSES, Howard R. Six, Arthur L. Frank, David Moffett and James Robinson. Baylor College of Medicine, Houston, TX 77030 and Yale University, New Haven, CT 06510

Peripheral blood lymphocytes from two adult volunteers were inoculated with EB virus and placed in microtiter plates under cloning conditions. Secretion of antibody to type A influenza virus was detected in up to 5% of wells at 3 to 5 weeks of incubation. Of 46 potential monoclones tested early, 16 bound only to H3N2 viruses and 13 of these were found to react with purified hemagglutinin (HA) protein. The HA specific monoclonal IgG antibodies were highly crossreactive when tested against 5 H3N2 variants representing the 1968-1981 time interval. All 13 monoclones reacted with at least 3 variants and 5 monoclones reacted with all 5 variants. One HA specific line (clone 4) and 3 of its subclones produced antibody for more than 9 mos. For subclone 4B, 0.5 mg of specific antibody was obtained from 125 ml of culture fluid by affinity chromatography. Competition for binding sites on the A/Texas/77-HA performed with radiolabeled subclone 4B and 8 other HA-specific monoclones indicated that at least two distinct antigenic sites were recognized by these antibodies. Two of 7 mouse monoclonal antibodies specific for the HA of A/Texas/77 were able to partially compete for the antigenic site recognized by subclone 4B. These data indicate that antibodies from man and mouse bind to regions on the HA that share a spatial relationship, but those obtained, thus far, from man exhibit greater crossreactivity than those derived from mice. The latter result suggests that differences in fine specificity or avidity may exist between HA specific antibodies from the two species.

1863 HUMAN GAMMA-INTERFERON PRODUCTION AND ITS RELATION TO INFLUENZA VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTE ACTIVITY, Yasuko K Yamada, Anthony Meager and Francis Ennis. University of Massachusetts Medical School, MA 01605 and NIBSC, London

We previously reported that acid labile IFN, which is known to be gamma-IFN, was produced in the human lymphocyte cultures after exposure to influenza virus-infected autologus stimulator cells. We recently developed a immunoradiometric assay for human alpha- and gamma-IFNs. The results with this assay were correlated with the results of bioassay. Donors could be divided into 2 groups based on types of IFN produced during stimulation. Some produced mainly gamma-IFN and others produced both alpha- and gamma-IFNs. Donors whose lymphocytes showed high levels of cytotoxic T lymphocyte (CTL) activity to influenza virus-infected autologus target cells (more than 30% specific lysis) generally produced large amounts of gamma-IFN (more than 100 IU). During the 6 days of stimulation, the amount of gamma-IFN in culture fluids gradually increased, especially in the last few days, along with the increment of CTL activity, but the amount of alpha-IFN did not increase after day 1. These observations suggest that influenza virus induced gamma-IFN production may be correlated with the induction of CTL activity. We developed cell lines using TCGF after induction of influenza virus-specific CTL activity. Gamma-IFN is produced by these cell lines following repeated stimulation by virus-infected autologus stimulator cells.

1890 EFFECTS OF IMMUNIZATION WITH ANTI-IDIOTYPE ANTIBODY ON ANTI-INFLUENZA VIRUS IMMUNE RESPONSES OF MICE. Jerome L. Schulman, Michael A. Reale, Thomas M. Moran, Marc Monestier and Constantin A. Bona. Mount Sinai School of Medicine, Dept. of Microbiology, New York, New York 10029.

Using both syngeneic polyclonal and monoclonal anti-idiotype antibodies, we have demonstrated shared idiotopes on Balb/c monoclonal antibodies specific for PR8(H1), and X-31(H3) hemagglutinins. Furthermore, we have detected the expression of these idiotopes in 2^{o} polyclonal responses of Balb/c mice immunized either with PR8 or X-31 influenza viruses and have demonstrated that the major component of this idiotype positive antibody is specific for viral HA or NA.

We have investigated the possibility that manipulation of the immune system by immunization with anti-idiotype antibodies might generate or prime for more cross-reactive anti-influenza immune responses. Mice were immunized with purified monoclonal anti-idiotype antibodies prepared against PR8 or X-31 specific monoclonal anti-HA antibodies and then were boosted with X-31 or PR8 virus. In addition, we have examined the effects of anti-idiotype immunization on immune responses following infection.

In separate experiments, we have studied the effects of idiotype suppression (immunization with repeated larger doses of anti-idiotype antibody) on immune responses to influenza virus.

Control through Use of Live, Attenuated Vaccines

1855 COMPARISON OF IMMUNE RESPONSES AND EFFICACIES OF INACTIVATED AND LIVE VIRUS VACCINES, Mary Lou Clements, Robert F. Betts, and Brian R. Murphy, University of Maryland School of Medicine, Baltimore, 21201; University of Rochester Medical Center, Rochester, NY; and National Institute of Allergy and Infectious Diseases, Bethesda, MD

The immunogenicity of two live attenuated cold-adapted (ca) influenza A reassortant viruses were compared in seronegative (HAI titer $\leq$ 1:8) adults with those to the licensed inactivated vaccine. In the first study, 94% of vaccinees receiving inactivated subvirion virus by the parenteral route had significant rises in serum HAI antibody, but only 31% showed nasal wash secretory IgA responses. In contrast, only 44% of the vaccinees receiving ca A/Washington/897/80 (H3N2) virus intranasally had serum HAI rises, whereas 69% had nasal IgA responses. Serum neuraminidase inhibiting antibody responses were similar for each group.

In the second study, isotype-specific antibody to hemagglutinin was measured by ELISA in serum and nasal wash specimens from 114 seronegative adults vaccinated with ca A/Washington/80 (H3N2) or A/California/10/78 (H1N1) virus intranasally or with inactivated vaccine parenterally. Live and inactivated viruses elicited serum IgA responses in 83% and 96% of vaccinees, and serum IgG responses in 72% and 100%, respectively; these antibody levels remained elevated for at least 6 months. Inactivated virus stimulated nasal IgG responses in significantly more vaccinees than did live virus (94% vs 59%, $P<0.01$). In contrast, only 38% of inactivated virus vaccinees had nasal IgA responses as compared with 83% of live virus vaccinees ($p<0.05$). Surprisingly, nasal IgG levels remained elevated in 65% of inactivated vaccinees for at least 6 months while nasal IgA levels remained elevated in 52% of live virus vaccinees.

To determine the short-term efficacy of these vaccines, vaccinees and unvaccinated seronegative adults were challenged intranasally with homologous wild-type virus 5 to 8

weeks after vaccination. Live virus vaccine completely protected against illness (16/16) whereas inactivated vaccine in 16 vaccinees provided only 72% protection. Wild-type virus was recovered less often in the live virus vaccinees (13% vs 63%) and 1000 times less virus was shed. This striking reduction in viral replication suggests that transmission may be more efficiently interrupted with live than with inactivated virus vaccination.

To determine the duration of immunity conferred, live (32) and inactivated (32) virus vaccinees and 42 unvaccinated controls were challenged with homologous wild-type influenza A 6-7 months after vaccination. Both types of vaccine gave comparable protection against systemic or febrile illness: 91% efficacy for the live virus vaccine and 75% efficacy for the inactivated virus vaccine. These data suggest that live attenuated ca viruses are promising vaccine candidates for an alternative to inactivated influenza A vaccines.

1864 FIELD TRIALS AMONG COLLEGE STUDENTS WITH LIVE ATTENUATED INFLUENZA VIRUS VACCINES, Robert B. Couch, John M. Quarles, Thomas R. Cate, and John M. Zahradnik, Influenza Research Center and Departments of Microbiology and Immunology, Baylor College of Medicine and Texas A&M University, Houston and College Station, Texas.

A series of clinical studies were performed to evaluate the utility of attenuated cold reassortant (CR) influenza virus vaccines for prevention of influenza. Closed challenges revealed type A CR vaccines to be highly immunogenic and to induce protection against artificial challenge similar to that induced by natural infection and inactivated vaccine.

In order to facilitate evaluations, A/USSR CR vaccine was tested for safety, immunogenicity, and transmissability in an open trial among college roommate pairs with one receiving vaccine and the other placebo. Illness was similar in vaccine and placebo groups and no transmission occurred. Three successive open field trials were then performed in this population as listed:

Vaccine trials	No. Vol.	No. doses	Result
monovalent A/USSR/77 (H1N1) CR vs placebo	1,400	1	safe,? protective
monovalent A/Alaska/77 (H3N2) CR vs placebo	640	1	safe, protective
bivalent A/Alaska/77 (H3N2) CR-A/Calif/78 (H1N1) CR vs trivalent inactivated vs placebo	600	2	safe, protective

The trials revealed the live vaccines to be safe for use in open populations of active, healthy young adults and little or no transmissability occurred. No overall protection was detected in the USSR trial but this was attributable to respiratory illnesses not shown to be caused by influenza viruses. Influenza virus was associated significantly with lower respiratory illness (LRI) and calculated protection for LRI was 36% among susceptibles and 53% among persons with an antibody response after vaccine. Immunogenicity results suggested a second dose of vaccine was needed.

Efficacy in the monovalent A/Alaska trial was estimated as 51% for infection determined by antibody response, 60% for infection-associated respiratory illness and 91% for infection-associated respiratory illness among those who had responded to vaccine.

The bivalent CR-inactivated vaccine trial was performed among students with low or absent serum antibody to both viruses. Efficacy was similar for both vaccines for any respiratory illness (41-46%), but greater for inactivated vaccine for infection and infection-related illness determined by serum antibody responses. In a preliminary evaluation of duration of protection, CR vaccine appears more efficacious two years after vaccination against natural infection with heterotypic H1N1 virus.

Thus, a series of clinical field trials with live attenuated cold-recombinant vaccines given intranasally have shown these vaccines to be safe for use in open, active young adult populations and to induce significant protection against naturally occurring influenza.

1865 PROSPECTS FOR TYPE B LIVE ATTENUATED INFLUENZA VIRUS, H. F. Maassab, D. C. DeBorde, A. Donabedian and C. W. Smitka, Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109.

The attenuation of type A influenza virus for use in man was accomplished by adapting the virus (A/AA/6/60-H2N2) to grow at the suboptimal temperature of 25°C (cold-adapted=ca). The development of a live virus vaccine is based on the identification of this ca "Master" strain and its use as a donor of attenuated gene(s) during reassortment with relevant wild-type (wt) strains. The same approach was used in the development of attenuated live vaccine to type B influenza virus. Derivation of type B cold-adapted (ca) "Master" strain (B/AA/1/66) was done by stepwise adaptation of the virus to yield maximal infectious titer at 25°C. It was also found that to obtain maximum yields, the type B line required fewer passages at the intermediate temperatures than type A influenza virus. The designated cold-adapted "Master" strain of B/AA/1/66 exhibited the genetic markers of ca and ts (shut-off temperature (37°C), was attenuated when tested in ferrets and man, and was genetically stable. In addition, the ca-B/AA/1/66 comes close to having mutations in every gene similar to the successful ca-A/AA/6/60 donor virus. Limited type B cold reassortant clones were derived and tested in man and found to be attenuated and genetically stable. The genotype profile for type B influenza virus vaccine necessary to provide reproducible attenuation will be established. A library of reassortants has been developed to study the genetic basis of cold adaptability, temperature sensitivity, and virulence for a pair of type B influenza virus strains: the avirulent, cold-adapted B/AA/1/66 and the virulent wild-type B/HK/72. The library includes single gene reassortants of all ca-B/AA/1/66 gene segments except RNA 4 (NP) and four single HK gene reassortants. A subset of the library has been analyzed for the association of plaquing phenotypes with particular gene segments. The functional protein coded by RNA segment 3 of ca-B/AA/1/66 was responsible for the expression of the ts phenotype, whereas, the ca phenotype was conferred by any of the single AA genes tested. Data will be presented concerning in vivo virulence of single gene reassortants and their parental strains in animals.

Control through Use of Antivirals and Alternative Measures

1866 RIMANTADINE PROPHYLAXIS OF CHILDREN, Richard Clover, M.D., Christian Ramsey, Jr., M.D., Steven Crawford, M.D., Troy Abell, Ph.D., Univ. of Okla., Okla. City, OK 73190

With recent studies suggesting that children are the main introducers of influenza infections into families, a placebo controlled, double blind randomized trial studying the prophylactic effectiveness of rimantadine in children on the transmission of Influenza A infections within families was undertaken. One hundred and forty-five volunteers from thirty-five families participated in this study during a naturally occurring outbreak of Influenza A H_1N_1 infection. All the children between the ages of 1 and 18 within each family received either placebo or rimantadine at 5mg/kg/day (maximum 150 mg/day) in a single daily dose for a five week period during the 1983-84 influenza season.

In the children, influenza infection, defined as a positive viral throat culture or a fourfold antibody titer rise, occured in 31.7 percent in the placebo group and 2.9 percent in the rimantidine group (P=.001). Clinical illness with laboratory evidence of influenza infection occurred in 17.0 percent of the placebo group and none in the rimantadine group (P<.01). Rimantadine was well tolerated by the children with no significant difference in reported side effects between the placebo and rimantadine groups. In the adults, influenza A infection occurred in 19.0 percent in those whose children where receiving a placebo and 8.8 percent in those adults whose children were receiving rimantidine 2.2 (P=0.2). The incidence in clinical illness in the adults was not statistically different between these two groups. On the basis of this study, rimantadine prophylaxis of children appears to be an effective method to prevent Influenza A infection in children. Even though there is a small decrease in the incidence of Influenza A infections in adults, further studies are needed to demonstrate the effects of rimantadine prophylaxis of children on the incidence of Influenza A infection in their parents.

1867 AMANTADINE AND RIMANTADINE: PROPHYLAXIS AND THERAPY OF INFLUENZA A IN ADULTS, Raphael Dolin, Infectious Disease Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Amantadine hydrochloride and rimantadine hydrochloride are antiviral compounds with demonstrated in vitro and in vivo effectiveness against influenza A virus. Viruses of all subtypes of influenza A virus have been found to be sensitive to the above compounds, although rimantadine has been noted to be 2-4 times more active against certain strains. Amantadine and rimantadine have been shown to be effective in the prophylaxis of influenza A in both experimentally induced and naturally acquired disease in a variety of populations. Investigators in the Soviet Union have reported that rimantadine is somewhat more effective than amantadine, but other studies have reported generally comparable chemoprophylactic efficacies of the two drugs, with efficacy rates of 70-90% in prevention of influenza A associated illness. Both drugs have a more profound effect on reduction of clinical illness than on infection, which is a potentially desirable feature of chemoprophylaxis. In recent studies, self-limited, but occasionally troublesome central nervous system side effects have been associated with amantadine at doses of 200mg/day, generally at a rate of 5-10% of recipients, while rimantadine has been non-reactogenic at this dose. Recently, the prophylactic effect of rimantadine has also been demonstrated in a placebo-controlled trial in 105 elderly nursing home residents, in which rimantadine reduced the rate of influenza-like illness by 63%. The effect of rimantadine was observed primarily in individuals who had previously received influenza A vaccination, suggesting that rimantadine and vaccination may confer additive protection. Of importance, rimantadine was also generally well tolerated in this elderly population.

Treatment with either amantadine and rimantadine has also been demonstrated to be efficacious in the therapy of experimentally induced and naturally occurring influenza A infection, in reducing the severity and duration of influenza-like illness. However, controlled studies have been carried out almost exclusively in mild self-limited disease young adults, and to a lesser extent in children. Virtually no controlled data are available regarding the effect of either amantadine or rimantadine in the treatment of influenza in high risk individuals or in the therapy of established complications of influenza, such as pneumonia. This remains an important area in which data from large scale, controlled studies are still required.

1. Zlydnikov DM, Kubar OI, Kovaleva TP, Kamforin LE. Study of rimantadine in the USSR: A review of the literature. Rev Infect Dis 3:408-21, 1981.
2. LaMontagne JR, Galasso GJ. Report of a workshop on clinical studies of the efficacy of amantadine and rimantadine against influenza virus. J Infect Dis 138:928-31, 1978.
3. Dolin R, Reichman RC, Madore HP, et al. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. NEJM 307:580-84, 1982.

1868 ANTIVIRAL TREATMENT OF RESPIRATORY INFECTIONS IN PEDIATRICS Caroline B. Hall, John T. McBride, Christine L. Gala, Raphael Dolin, Departments of Pediatrics and Medicine, University of Rochester Medical Center, Rochester, NY 14642

Two new antiviral agents, rimantadine and ribavirin, currently hold promise for the treatment of acute respiratory infections with influenza A virus and respiratory syncytial virus (RSV). Rimantadine, an analog of amantadine, has been shown in controlled studies in adults to be beneficial in both the prophylaxis and treatment of influenza A infections and is accompanied by fewer side effects than with amantadine(1). The use of rimantadine in children, however, had not been studied. Thus, during the 1983 outbreak of influenza A/H3N2 infections, we evaluated in a controlled study the effect of rimantadine in the treatment of influenza in children compared to treatment with acetaminophen(2). Children with influenza-like illness were randomly assigned in a double blind fashion to receive either rimantadine or acetaminophen for five days. During the first three days of treatment the decline in fever and scores for daily symptoms and severity of illness was significantly greater in children receiving rimantadine. The proportion of children shedding virus and the amount of virus shed were also significantly less in the rimantadine group during the first two days. However, after day 4 the reverse was true; on days 6 and 7 viral shedding was significantly greater in the rimantadine group.

Ribavirin is a synthetic nucleoside possessing antiviral activity in vitro against both RNA and DNA viruses. Ribavirin administered by small particle aerosol has been shown to be beneficial in the treatment of both influenza A and influenza B infection in adults(3,4). Ribavirin has been administered by small particle aerosol to young adults with experimental RSV infection(5). Treated volunteers demonstrated less systemic symptoms, fever and viral shedding compared to the placebo-treated group. No toxicity occurred by any examination, including sequential pulmonary function tests. Ribavirin aerosol therapy of infants hospitalized with lower respiratory tract disease was next evaluated in two controlled, double blind studies(6,7). In both studies treated infants showed significantly more rapid improvement, including in one study a significantly greater change in the mean arterial oxygen saturation. Ribavirin aerosol treatment of other types of acute respiratory infections in the young needs to be evaluated. Its use in the overwhelming respiratory viral infections of infants with severe combined immunodeficiency disease has in a few cases appeared promising(8,9).

References:
1. Dolin RM et al:NEJM 307:580-584,1982.
2. Hall CB et al:Ped Res 18:276A,1984.
3. Knight V et al:Lancet 2:945-949,1981.
4. McClung HW et al:JAMA 249:2671-2674,1983.
5. Hall CB et al:JAMA 249:2666-2670,1983.
6. Hall CB et al:NEJM 308:1443-1447,1983.
7. Taber LH et al:Pediatrics 72:613,1983.
8. Gelfand EW:Lancet 2:732-733,1983.
9. McIntosh K et al:AJDC 138:305-308,1984.

1869 RIBAVIRIN AEROSOL TREATMENT OF INFLUENZA, Vernon Knight, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030.

This report will review the principles and methodology of the use of small-particle aerosol in the treatment of influenza. The equipment currently in use and the basis for estimating dosage will be described. A summary of several years of study of infections caused by several different influenza A viruses and influenza B virus will be presented. The clinical and laboratory findings in treated and control patients that reveal therapeutic effect will be described. Clinical and laboratory studies have shown no evidence of intolerance or toxicity but details of the effect of influenza on circulating white blood cell counts revealed by these studies will be presented. Information concerning the effect of ribavirin aerosol in the treatment of influenzal pneumonia will also be reviewed.

Antigen Structure and the Production of Antigens by In Vitro Methods

1870 PROSPECTS FOR ENHANCEMENT OF IMMUNOGENICITY OF SYNTHETIC ANTIGENS BY MURAMYL PEPTIDES Louis A. Chedid and Françoise M. Audibert, Immunothérapie Expérimentale, Institut Pasteur, Paris France.

Chemically synthesized peptides copying sequences of bacterial, viral or parasitic structures can produce antibodies which can recognize the native protein and even in some cases can be protective (1-4). To become immunogenic these peptides must generally be coupled to protein carriers and also administered with adjuvants. Thus, high antibody titers can be obtained by administering in saline a conjugate (containing the antigen coupled to tetanus toxoid) with muramyl peptides (MDP) which are synthetic adjuvants (5). One MDP analogue, Murabutide, has been shown to be devoid of detectable side effects and, in association with conventional vaccines, is currently undergoing clinical trials (6).

However, clinical use of carriers, even such as tetanus toxoid, presents several limitations and therefore efforts were made to produce totally synthetic vaccines. Previous studies have demonstrated that efficient secondary immunizations can be achieved using a synthetic streptococcal peptide following primary stimulation by the streptococcal protein with Murabutide (7). As will be reported, protective antibodies have also been obtained using MDP derivatives with other bacterial, viral or parasitic synthetic immunogens without preliminary exposure to the natural antigen. In a different system, a linear conjugate containing the decapeptide hormone LH-RH and MDP-Lys was also shown to be effective, producing antibodies and immunological castration (8).

Will also be reported synthetic polyvalent vaccine constructs which can elicit high levels of antibodies recognizing four different specificities. Therefore, under appropriate conditions, immunodominance between covalently linked peptides of different specificities can be avoided.

1. Audibert F, Jolivet M, Chedid L, Alouf JE, Boquet P, Rivaille P, Siffert O. Nature 1981, 289, 593-594.
2. Fraser HM, In Hearn JP, Immunological Aspects of Reproduction and Fertility Control. MTP Press Ltd., Falcon House, Lancaster, 1980, pp. 143-171.
3. Sutcliffe JG, Shinnick TM, Green N, Lerner RA, Science 1983, 219, 660-666.
4. Leclerc C, Morin A, Chedid L, In Thompson RA, Rose RN, Recent Advances in Clinical Immunology Churchill Livingstone, Edinburgh, 1983, 3, 187-204.
5. Ellouz F, Adam A, Ciorbaru R, Lederer E, Biochem.Biophys.Res.Com. 1974, 59, 1317-1325.
6. Chedid LA, Parant MA, Audibert FM, Riveau GJ, Parant FJ, Lederer E, Choay JP, Lefrancier PL Infect. Immun. 1982, 35, 417-424.
7. Jolivet M, Audibert F, Beachey EH, Tartar A, Gras-Masse H, Chedid L, Biochem. Biophys. Res. Com. 1983, 117, 359-366.
8. Carelli C, Audibert F, Chedid L, Gaillard J, Proc.Natl.Acad.Sci. USA 1982, 79, 5392-5395.

1871 THE INTERACTIONS OF THE INFLUENZA HA WITH ANTIBODIES, Don C. Wiley, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, Marcel Knossow, Laboratoire de Physique, Centre Pharmaceutique, Chatenay-Malabry, France, Rodney S. Daniels and John J. Skehel, National Institute of Medical Research, Mill Hill, The Ridgeway, London NW7 1AA, England

The nature of sites on the influenza HA which are recognized by mouse monoclonal antibodies and which have undergone antigenic variation in response to human antisera (1) will be discussed. X-ray crystallographic structures of single amino acid substitution mutants selected by their ability to escape neutralization by monoclonal antibodies have been determined to define the location of antibody binding and the extent of structural alteration required to escape neutralization (2). Current efforts to calculate the structure of such single amino-acid mutants (Shih, Brady and Karplus, unpublished) will be compared to the X-ray results. The coordinates of the 1968 Hong Kong HA trimer has been refined by restrained least squares method to an R factor of 22%, from which certain generalizations concerning the nature of antigenic sites can be made.

(1) Wiley, D. C., Wilson, I. A. and Skehel, J. J. "Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation." Nature 289, 373-378 (1981).

(2) Knossow, M., Daniels, R., Skehel, J. J. and Wiley, D. C. "Three-dimensional structure of an antigenic mutant of the influenza virus haemagglutinin." Nature 311, 678-680 (1984).

Specificity of the Immune Response at the Clonal Level

1872 T-CELL REPERTOIRE: SPECIFICITY OF CYTOTOXIC T-CELL CLONES FOR INFLUENZA VIRUS, Brigitte A. Askonas, Pietro Pala and David C. Wraith, NIMR,The Ridgeway, London NW7.
Both in man and mouse, a majority ot T-cells from donors primed by infection do not discriminate between serologically distinct type A influenza viruses, while a minority population is virus variant specific. We are examining the repertoire of mouse cytotoxic T-cells (using cloned Tc as well as limiting dilution clones) for recognition of target cells that are (a) infected with recombinant influenza viruses and (b) transformed by genes coding for individual viral proteins. The latter target cells were kindly provided by Dr. A. Townsend. In H-2^k mice a very low proportion of T-cells from mice primed by infection with PR8 or X31 see HA or NA; a higher proportion see NP transformed cells and these can be A virus cross reactive. However additional epitopes need to be defined since several A virus cross reactive Tc clones have not been so far mapped to any of the influenza proteins tested.

1873 CYTOTOXIC T CELL RECOGNITION OF INFLUENZA VIRUS GENE PRODUCTS, Jack R. Bennink, Jonathan W. Yewdell, Geoffrey L. Smith, and Bernard Moss, Wistar Institute, Philadelphia, PA 19104 and The National Institutes of Health, Bethesda, MD 20205
Studies examining the recognition of influenza virus gene products have been done using vaccinia virus recombinants containing DNA copies of individual influenza virus genes. To date, recombinants containing either the A/PR8 or the A/JAP hemagglutinin (H1-VAC and H2-VAC), an A/PR8 nucleoprotein (NP-VAC), or an A/PR8 matrix protein (M-VAC) have been examined. Results indicate that cytotoxic T cells (CTL) capable of recognizing the hemagglutinin molecule are almost entirely subtype specific. Although a small degree of cross-reactivity has been observed betwwee H1 and H2 hemagglutinins it is clear that the hemagglutinin in no way accounts for the major cross-reactive specificity observed in anti-influenza virus CTL populations. Experiments with NP-VAC indicate that the nucleoprotein does serve as a major cross-reactive target structure. Cold target inhibition assays indicate that there may be other cross-reactive determinants on additional molecules. CTL, however, appear not to recognize the matrix protein, since M-VAC neither primes nor stimulates influenza specific CTL and target cells infected with M-VAC are not lysed by anti-influenza CTL populations.

1874 MURINE T CELL RESPONSE TO INFLUENZA HEMAGGLUTININ, Lorena E. Brown, Jacqueline M. Katz, Rosemary Ffrench, E. Margot Anders and David O. White, University of Melbourne, Parkville, Vic., 3052 Australia.

We have examined the recognition of influenza virus hemagglutinin (HA) by T lymphocytes by assaying the proliferative response of virus primed T cells to purified HA, HA_1 and HA_2. Proliferating T cells differed from B cells in their recognition of HA in two ways. 1) T cells from mice primed to one strain of type A influenza virus cross reacted with other purified HA's, not only of the same subtype as the priming virus but also of serologically distinct subtypes of A (but not B) virus. 2) A large proportion of the cross reactive response was directed against HA_2.

T cells at the clonal level recognizing the HA were isolated by limit dilution from lines stimulated with H3 virus and were found to recognize either H3 specific determinants or determinants shared by H3 and H2 HA's. Both types responded to determinants on HA_1. Of these, one specific and one cross reactive clone were further characterized and found to be $L3T4^+$ and required presentation of HA in association with I-E on macrophages. These clones were able to help influenza primed B cells make anti-HA Ab in response to virus.

1875 RECOGNITION OF THE VIRAL NUCLEOPROTEIN AND HAEMAGGLUTININ BY INFLUENZA A SPECIFIC CYTOTOXIC T CELLS. Alain R Townsend, Frances M Gotch, Andrew J McMichael, John Davey and George G Brownlee, Nuffield Dept. of Medicine, John Radcliffe Hospital, Oxford.

In recent work we have used recombinant A viruses to show that the viral nucleoprotein (NP) gene plays a role in recognition of target cells by influenza specific cytotoxic T lymphocytes (CTL). This has been confirmed and extended by examining CTL recognition of L cells co-transfected with an expression vector containing the A/NT/60/68 (H3N2) NP gene and a cosmid expressing the Class I restriciton element D^b. A major population of A virus cross reactive CTL from C3H and C57BL/6 mice can be demonstrated that recognise NP transfected target cells. Recognition of NP is A virus specific and H-2 restricted. Experiments with human CTL are in progress. Comparative experiments have been done using a similar vector expressing the haemagglutinin (HA) gene from A/PR/8/34 (H1N1). No evidence could be found in the C3H mouse for recognition of expressed HA by fully A virus crossreactive CTL, although a subpopulation of CTL with restricted specificity for H1 and H2 haemagglutinin was confirmed. In view of the fact that NP was recognised more efficiently by polyclonal CTL than the HA, it became of interest to investigate how this non integral membrane protein acts in transfected or infected cells to induce specific recognition by T cells. Experiments in progress involve the use of in vitro mutated genes to localise the regions of the NP and HA molecules responsible for recognition.

1876

STIMULATION OF INFLUENZA VIRUS SPECIFIC CYTOTOXIC T CELLS WITH PURIFIED VIRAL PROTEINS. David C. Wraith & Brigitte A. Askonas, N.I.M.R., Mill Hill, London. U.K.

Influenza specific cytotoxic T (Tc) cells can protect against infection. Furthermore the majority of Tc are cross-reactive within a virus type. Current evidence suggests, however, that infection with live virus is required to stimulate Tc in vivo. We have extended these findings by purifying influenza proteins and comparing their ability to stimulate Tc in mice. Haemagglutinin H3, whether as a soluble fragment, rosettes or incorporated into liposomes, neither stimulated Tc in vivo nor induced a secondary response in vitro. On the other hand, influenza virus A nucleoprotein, when prepared by a mild detergent procedure, stimulated A virus cross-reactive Tc in vivo. As some of our Tc clones do not recognise nucleoprotein we are currently testing other viral proteins as well as studying the immune response to nucleoprotein and its potential as a vaccine.

1877 INDUCTION OF INFLUENZA VIRUS SUBTYPE-SPECIFIC CTL RESPONSE USING A POLYPEPTIDE PRODUCED IN E. COLI. Akio Yamada, James F. Young,* and Francis A. Ennis. Division of Infectious Disease, Department of Medicine, University of Massachusetts Medical School. *Molecular Genetics Division, Smith Kline and French Laboratories, 1500 Spring Garden Street, Philadelphia, PA 19101.

In order to clarify the antigen(s) recognized by influenza virus-specific cytotoxic T lymphocytes (CTL), we examined the abilities of several viral polypeptides prepared in E. coli to induce the secondary CTL response in vitro. A hybrid protein containing a portion of non-structural protein (NS_1) and the HA_2 subunit of hemagglutinin of A/PR/8/34 (H1N1) was shown to induce a secondary response which is virus subtype (H1) specific and is restricted by major histocompatibility antigen(s). Using a recombinant virus strain, the antigenic site responsible for the recognition appears to be on the HA_2 portion. This protein also induced virus subtype-specific memory CTL in vivo when inoculated into mice with Freund's complete adjuvant. The frequency of CTL precusors reactive with this protein was 1/73,177, whereas that reacting with virus-infected cells was 1/15,111. These results indicate that this E. coli-derived HA_2 containig polypeptide induces subtype-specific CTL both in vitro and in vivo, and that about 20% of the CTL precursors recognize subtype-specific determinant(s) on HA_2 protein.

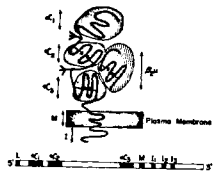
Immune Recognition of Influenza Virus Antigens

1878 HUMAN T CELL RECOGNITION OF INFLUENZA VIRAL ANTIGENS AND CLASS II MHC GENE PRODUCTS, Jonathan R. Lamb and David D. Eckels*, ICRF Tumour Immunology Unit, University College London, London WC1E 6BT and * Immunogenetics Research Section, Blood Center of Southeastern Wisconsin, Milwaukee, WI 53233.

The induction of T cell responses involves the recognition of extrinsic antigen in association with determinants encoded by genes of the major histocompatibility complex (1). By isolating human T cell clones induced with influenza A virus (H_3N_2) it has been possible to analyse both viral antigen recognition and restriction specificity. Initial studies revealed that T cell clones recognized a variety of viral antigens including haemagglutinin, neuraminidase, matrix protein and nucleoprotein (2). Although most clones responded to antigen when presented by DR matched accessory cells, some were restricted by non DR gene products (3). Subsequent analysis has revealed that HLA-DP (4) and DQ (5) class II molecules can function as restriction elements in the recognition of influenza viral antigens. However, there was no apparent correlation between antigen specificity and genetic restriction. With synthetic peptides of the HA molecule it was possible to demonstrate an immunodominant epitope recognized by most HA reactive clones (6). Genetic analysis suggested that two clones recognizing the same epitope has identical restriction pattern although it could not be correlated with any classic D region specificity (5).

1. Klein J. and Nagy, Z.A. Adv. Can. Research 37, 233 (1982).
2. Lamb, J.R. et al. J. Immunol. 128, 1428 (1982).
3. Eckels, D.D. et al. Human Immunology. 4, 313 (1982).
4. Eckels, D.D. et al. Nature 301, 716 (1983).
5. Eckels, D.D. et al. Immunogenetics 19, 409 (1984).
6. Lamb, J.R. et al. Nature 300, 66 (1982).

1879 T CELL RECOGNITION ELEMENTS ON CLASS I MHC GENE PRODUCTS WHICH RESTRICT ANTIVIRAL CTLS. Carol S. Reiss, Steven J. Burakoff, J.G. Seidman, and Cornelis Murre. Dana-Farber Cancer Inst., and Depts. of Genetics and Pathology, Harvard Medical School, Boston, Mass., 02115.



Functional epitopes on Class I major histocompatibility (MHC) antigens have been evaluated using techniques of both molecular biology and cellular immunology. Genes encoding H-2 D^d and H-2 L^d have been cloned and for some studies, portions of the DNA have been recombined by the technique of "exon shuffling". These genes and their exchanged hybrid products have been used to transfect mouse L cells by the calcium phosphate method; cloned cell lines have been developed and evaluated immunologically.

Using these cell lines, monoclonal antibodies have been found to bind to epitopes on all three extracellular domains. Cytolytic T lymphocytes (CTLs) specific for influenza, vesicular stomatitis, and Sendai viruses, and alloantigens were generated in vitro from murine splenocytes and used to assess the transfected L cells as suitable targets for lysis. Studies with bulk populations were confirmed with cloned CTLs.

Several observations were made. For all CTL systems investigated, all of the polymorphic restriction sites were found to map to the two most external domains, known as $\alpha 1$ and $\alpha 2$. No polymorphic sites could be attributed to the $\alpha 3$ domain of class I MHC antigens. Recombinants which separated the $\alpha 1$ and $\alpha 2$ domains served as effective targets for appropriate CTL lysis. Molecules of H-2 L^d which lacked the cytoplasmic portion served as effective targets for allorestricted, influenza and Sendai virus restricted CTLs, but were poor targets for VSV-restricted CTLs. Cells which secreted H-2 L^d molecules stimulated the induction of CTLs, but failed to serve as targets for lysis. Work is in progress on the biologic function of molecules which have the external portion of H-2 L^d and the transmembrane and intracellular portion of immunoglobulin.

References: Reiss, et al, Proc. Natl. Acad. Sci. (USA) 80:2709-2712, 1983.
Murre, et al, Nature (London) 307:432-436, 1984.
Murre, et al, J. Exp. Med. 160:167-178, 1984.

Synthesis of Influenza Virus Components In Vivo and In

1880 THE FUNCTIONAL DOMAINS OF INFLUENZA NEURAMINIDASE, Timothy J. Bos, Alan R. Davis, and Debi P. Nayak, U.C.L.A. School of Medicine, Los Angeles, CA 90024

The influenza neuraminidase (NA) has been studied extensively at the molecular level because of its unusual structural properties and because of its importance in viral infectivity and pathogenesis. We have been concerned with defining the functional domains of NA as they relate to structure. To accomplish this, we have cloned the gene encoding NA from the strain A/WSN/33 and expressed it in CV1 cells using SV40 as a vector. By following the behavior of expressed proteins encoded by specifically altered NA cDNA's, we have been able to study the function of specific structural domains. Three domains were studied: the cytoplasmic tail, the hydrophobic region near the NH_2-terminus, and the active region. The cytoplasmic tail, located at the NH_2-terminus, has as yet an undefined function. Although highly conserved in sequence, this domain can be completely replaced by foreign amino acids with no effect on expression, glycosylation, or transport to the surface. The hydrophobic region near the NH_2-terminus is multifunctional, being important in translocation as well as anchoring. We have shown this region to act as an uncleaved signal for translocation across the rough endoplasmic reticulum by the cotranslational glycosylation of a chimeric protein consisting of the first 40 amino acids of NA followed in phase by hemagglutinin (HA) minus its own signal sequence. We are studying the active region by utilizing temperature sensitive mutants of NA which are thermolabile and which vary in substrate specificity at the restrictive temperature. We have cloned the NA gene from three of these mutants into the SV40 expression vector and are in the process of characterizing the ts lesion.

1881 A KARYOPHILIC SEQUENCE IN INFLUENZA VIRUS NP PROTEIN, John Davey, Alan Colman and Nigel J. Dimmock, University of Warwick, Coventry, U.K.

Influenza virus nucleoprotein (NP) synthesized in Xenopus oocytes after injection of cloned NP cDNA, enters and accumulates in the nucleus. Mutants lacking amino acids 327-345 of wild-type NP enter the nucleus but do not accumulate there to the same extent as the wild-type protein, suggesting that this region has a role in nuclear accumulation. This possibility is further strengthened by similar studies involving the production of fusion proteins in which various amino terminal sequences of the NP gene are fused to the complete chimpanzee α_1-globin sequence: when globin cDNA is injected into and expressed in oocytes the protein remains exclusively in the cytosol, however when the globin cDNA is fused to a portion of NP cDNA which includes the region encoding amino acids 327-345, the resulting fusion protein enters and accumulates in the nucleus. Fusion proteins lacking this region of the NP enter but do not accumulate in the nucleus.

1882 THE APPARATUS FOR TRANSCRIPTION AND REPLICATION OF INFLUENZA A VIRUS. Akira Ishihama, Atsushi Kato, Masakazu Hasegawa, Ryuji Fukuda, Kiyohisa Mizumoto and Kazufumi Shimizu, Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka 411, JAPAN

The isolation and characterization of RNA polymerase from influenza A virus indicate that NP-free RNPs, composed of eight RNA genomes and three P proteins, are still active in catalyzing both the endonucleolytic cleavage of capped RNA and the primer-directed RNA synthesis. This suggests that NP is not required at least for primary transcription. Several lines of evidence indicate that the RNA polymerase carries a proof-reading function, by which unfaithfully polymerized nucleotides are removed prior to further elongation.

By contrast, in vivo studies indicate that temperature-sensitive mutants carrying mutations in NS-1 gene are defective in the switch from primary to secondary transcription, implying that NP-1 is required for the functional conversion of RNA polymerase.

1883 IN VITRO SYNTHESIS OF FULL-LENGTH INFLUENZA VIRUS COMPLEMENTARY RNA. Juan Ortín, Lucía del Rio, José A. López de Turiso, Concepción Martínez and Esteban Domingo. Centro de Biología Molecular (CSIC-UAM). Cantoblanco. 28049 Madrid, SPAIN.

An in vitro system has been set up from microsomal fractions of MDCK cells infected by influenza virus, that is able to synthesize full-length messenger and complementary RNA. The synthesis could be stimulated 5-10 times by ApG. Most of the RNA product lacked poly A tails and was of positive polarity, as shown by filter hybridization to M13 strand-specific probes and by T1-fingerprinting. Electrophoresis on polyacrylamide-urea sequencing gels showed that poly A^- RNA was indistinguishable by size of virion RNA, while poly A^+ RNA contained poly A tails about 50 nucleotides long. The size of the poly A^- and poly A^+ RNAs was also compared by polyacrylamide gel electrophoresis of S1-treated ds-RNA derived by hybridization with unlabelled virion RNA. When poly A^- and poly A^+ HA and NP RNAs were analyzed by T1-fingerprinting after selection with 3'-terminal cloned DNA probes, poly A^- RNAs showed additional T1-oligonucleotides of predicted size, when compared to the corresponding poly A^+ RNAs, indicating the synthesis in vitro of full-length complementary RNA.

1884 EXPRESSION OF INFLUENZA VIRUS cDNA's IN MAMMALIAN CELLS, John W. Wallen[1,2] and James F. Young[2],
[1]University of Pennsylvania, Philadelphia, PA and
[2]Smith Kline and French Laboratories, Philadelphia, PA 19101

With the advent of recombinant DNA technology it is now possible to clone and express the influenza virus genes in both procaryotic and eucaryotic systems in order to study the structure and function of their encoded proteins. Using this technology we have constructed mammalian cell lines which constitutively express either the hemagglutinin (HA) or the nucleoprotein (NP) of A/PR/8/34 virus. The expression of these proteins was achieved with a bovine papilloma virus-based vector containing the neomycin resistance gene as a dominant selectable marker. The transcription of the influenza virus genes is under the control of the SV40 early promoter.
The expression vector constructs containing the influenza virus cDNA's were transfected into mammalian cells. G418 resistant colonies were isolated and screened for the expression of the influenza virus proteins by indirect immunofluorescence using monoclonal antibodies specific for the HA and NP proteins. Positive clones were subcloned to homogeneity and characterized. Western blotting and immunoprecipitation analysis of these cells demonstrated the production of proteins weith electrophoretic mobilities consistent with those synthesized by virus. In addition, the HA was expressed on the surface of these cells and the NP was observed in the nucleus. Experiments using these different cell lines to study the immunobiology and molecular biology of influenza viruses will be presented.

1891 INFLUENZA VIRUS M_2 PROTEIN IS AN INTEGRAL MEMBRANE PROTEIN EXPRESSED ON THE INFECTED-CELL SURFACE, Robert A. Lamb, Suzanne L. Zebedee and Christopher D. Richardson, Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston IL 60201 and Laboratory of Molecular Genetics, NINCDS, NIH Bethesda MD 20205

Studies on the cellular location of the influenza A virus M_2 protein have shown that it is expressed abundantly at the cell surface, and in addition to the hemagglutinin (HA) and neuraminidase (NA) it is a third virus-specific membrane protein. M_2 has an internal hydrophobic membrane anchorage domain and it associates with the same cellular membrane fractions as HA and NA. Trypsin treatment of infected cells and immunoprecipitation with antisera specific for the M_2 protein NH_2-terminus and a COOH-terminal region indicate that a minimum of 18 NH_2-terminal amino acids of M_2 are exposed at the cell surface. The first 10 NH_2-terminal residues are conserved in all avian and human strains of influenza A virus for which sequences are available. Antibodies can recognize the M_2 NH_2-terminus on the cell surface and therefore M_2 may be an infected-cell surface antigen. Properties of M_2 will be discussed that match the immunological observations for the elusive major target molecule on influenza A virus-infected cells for cross-reactive T cells.

In Vivo Efficacy of Viral Products, Immune Cells and their Products

1885 INDUCTION OF A VIRUS SPECIFIC IMMUNE RESPONSE BY AN ANTI IDIOTYPIC ANTIBODY, Hildegund C.J. Ertl and R.W. Finberg, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

A parainfluenza virus type I (Sendai virus) specific T helper cell clone was used to raise in syngeneic mice a monoclonal antibody. This antibody which was characterized as an IgM molecule bound to the T cell clone used for its induction and in addition bound to approximately 30% of all Sendai virus specific T cell clones/lines we have tested so far. It failed to bind to naive T cells or to T cells directed against an unrelated antigen and could thus be identified as an anti-idiotypic reagent which presumably recognizes a dominant idiotype of the Sendai virus specific T cell repertoire. This idiotype is mainly but not exclusively expressed on Lyt-1^+,2^- T cells.

The anti-idiotypic antibody was used to induce an anti-viral immune response in vivo. Upon i.p. injection of the antibody - which has no anti-viral activity - mice of different strains irregardless of their H-2 haplotype or IgH allotype generated : i) T cells which upon in vitro restimulation with Sendai virus lysed virus infected target cells; ii) T cells which upon adoptive transfer mediated a delayed type hypersensitivity response to Sendai virus; iii) Idiotypic antibodies with specificity for Sendai virus. Mice which were preimmunized with the antibody were protected against a subsequent inoculation of a lethal dose of Sendai virus.

1886 THE ROLE OF IgA SPECIFIC HELPER-T CELLS IN MURINE INFLUENZA VIRUS INFECTION, F.Y.Liew, Dept. of Experimental Immunobiology, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.

Mice previously infected with an aerosol of A/Rec 31 (H_3N_1) influenza virus were strongly protected against an aerosol challenge with A/Okuda (H_2N_2) influenza virus as judged by lung virus titres recovered two days after the challenge infection. Such heterotypic immunity was not achieved by priming with live Rec 31 virus injected i.v. or UV-inactivated Rec 31 virus administered s.c. together with $Al(OH)_3$ and saponin. The protection induced by respiratory infection with Rec 31 virus was specific for influenza A virus. An earlier study (Liew et al, Eur. J. Immunol. 1984, 14:350-356) has demonstrated that the preventive heterotypic immunity induced in this system was not correlated with specific serum HAI antibody titre or cross-reactive cytotoxic T cell reactivity, but occurred in parallel with the presence of cross-reactive IgA antibody in the lung washings. The heterotypic immunity also coincided with the early appearance of local specific IgA antibody (but not IgG antibody) which was significantly enhanced two days after challenge infection and peaked at day three. The heterotypic immunity with its parallel augmentation of local specific IgA response could be adoptively transferred by immune splenic and lymph node cells which expressed the L3T4 marker characteristic of helper T cells. It thus appears that activated, recirculating, IgA-specific helper T cells play a significant role in the protection against heterotypic influenza virus infection.

1887 HOMOLOGOUS AND HETEROLOGOUS IMMUNITY TO INFLUENZA A VIRUS INDUCED BY RECOMBINANTS OF THE COLD-ADAPTED MASTER STRAIN A/ANN ARBOR/6/60-CA, Gregory A. Tannock and Judith A. Paul, Faculty of Medicine, The University of Newcastle, New South Wales 2308, Australia

A suggested major advantage of the use of living attenuated influenza vaccines for the prophylaxis of influenza A is the capacity of these vaccines to induce effective immunity to different influenza viruses within the same sub-type. The effectiveness of heterotypic cross-protection was examined in a mouse model using outbred CSL mice over a period of 7 months. Mice were vaccinated intranasally using two doses of ca viruses with H2N2, H3N2 and H1N1 surface antigens. A vaccinating dose consisting of $3\overline{0}$ PD_{50} was used for each animal. Good homologous and heterologous protection against each virulent wild-type parental virus was noted up to 6 weeks. However, after this time the heterologous but not homologous protection induced by each of the ca viruses declined, suggesting that two mechanisms of immunity are involved.

1888 PROTECTION AGAINST INFLUENZA A VIRUS INFECTION IN MICE BY LIVE ATTENUATED VACCINES. MARTHA A. WELLS, SYLVESTER DANIEL, D. BRUCE BURLINGTON, AND GERALD V. QUINNAN. FDA, Bethesda, MD

It has been proposed that live influenza A virus vaccines are potentially superior to killed virus vaccine (KV) in that they may induce more durable and cross-reactive immunity. We compared cytotoxic T lymphocyte (CTL) responses and efficacy of live and KV vaccines. BALB/c mice were immunized to A/Bangkok/1/79 (H3N2) influenza with 0.1, 1 and 10 seroconverting doses of either KV, wild type virus (WTV), avian recombinant virus (ARV) or live cold recombinant virus (CRV) vaccines. Virus recovery from nasal mucosa was WTV > ARV > CRV. When immunized mice were challenged at 30 days with 1, 10, or 100 lethal dose 90%, of mouse adapted wild type virus, survival was found to be significantly enhanced (33% to 100%) at all challenge levels in mice immunized with WTV and KV whereas CRV immunized mice were protected against only the lowest challenge dose. In a second experiment, immunization with similar seroconverting doses of WTV and ARV demonstrated that ARV also protected against lethal challenge.

Stimulation in vitro of lymphocytes from mice previously immunized with WTV, KV, CRV or ARV vaccines induced CTL responses in all cases. Passive transfer of each preparation of CTL to athymic nude mice resulted in enhanced survival (40 to 92% vs 0%) and decreased pulmonary virus titer after challenge. These data demonstrate that ARV, CRV and WTV induced CTL and protective immunity proportionate to their growth in mice. KV also induced CTL precursors which could be passively transferred producing protective immunity.