



## CONFERENCE HIGHLIGHTS

### Welcome

Ellis Rubinstein, Chief Executive Officer, New York Academy of Sciences

- The NYAS will be launching in Fall 2003 a series of seminars in the hottest fields of science.
- Seminars and conferences will be presented on the Web with educational materials and other resources.
- This event is the start of an exciting partnership at the Academy to bring together industry and academic scientists.
- The SARS epidemic has brought out acts of heroism on the part of those on the front lines, including scientists, doctors, and journalists.

### Introductory Remarks

W. Ian Lipkin, Mailman School of Public Health, Columbia University, New York

- Dr. Lipkin phoned into this conference because he had contracted a fever and cough after a visit to China. Although expert consensus is that he does not have SARS, Dr. Lipkin remained in quarantine until May 25, 2003.
- Dr. Lipkin serves as a special advisor on SARS to the Ministries of Science and Technology and Health in China and is helping to coordinate international research efforts.

## Session I: Coronavirus Biology and Pathogenesis

Moderated by Scott Hammer

### Coronavirus Biology and Pathogenesis: Molecular Biology of Coronaviruses

Paul Masters, Wadsworth Center, NY State Dept. of Health, Albany

#### Virion Characteristics

- Coronaviruses are enveloped, positive-strand RNA viruses.
- Pleomorphic virions of 80-120 nm in diameter have a punched-in spherical appearance by negative-stained EM.
- There are characteristic 20nm surface projections on the envelope.
- The nucleocapsid is helically-symmetrical, unique among positive-strand viruses.
- Four basic structural proteins are encoded by the virus genome: spike (S), membrane (M), envelope (E), and nucleocapsid (N).

#### Infection and Disease Features

- Coronaviruses generally cause respiratory, enteric, and neurologic diseases.
- Infections are highly species-specific.
- Coronaviruses have been grouped by serological cross-reactivity into three groups; by genomic sequence homology; SARS is the prototype member of a fourth group.

#### Spike Protein

- S protein is a 150 kDa, type I membrane protein.

Columbia University DKV | New York Academy of Sciences | Mailman School of Public Health | NIAID



- Has a large N-terminal ectodomain, that is extensively N-linked glycosylated; a transmembrane domain; and short COOH-terminal endodomain.
- Oligomerizes into dimers or trimers that form the spikes.
- Binding to a receptor triggers fusing between viral envelope and a cellular membrane (plasma or endosomal) and internalization of the nucleocapsid into the cytoplasm.
- Can induce fusion of adjacent cells to form syncytia.
- Is the principle viral antigen, eliciting neutralizing antibody from the host.

## Membrane Protein

- M protein is a 25 kDa, triple membrane-spanning protein, with a short N-terminal ectodomain that is either N- or O-glycosylated, and a large COOH-terminal tail that interacts with the viral nucleocapsid.
- Most abundant viral protein and a major determinant of virion morphogenesis.
- Selects S protein and the genome for incorporation into virions during viral assembly.

## Envelope Protein

- E protein is an 8-10 kDa protein present in tiny stoichiometric quantities.
- Determines the site of viral budding, either ERGIC or Golgi.
- Expression of only M and E proteins leads to formation of virus-like particles and export from cells.

## Nucleocapsid Protein

- N protein is a 50 kDa protein with overall positive charge.
- The protein is overall basic, but the COOH-terminus is acidic.
- Central region binds to RNA; COOH-terminus binds to M protein during assembly.
- N may serve as a translational enhancer.

## The RNA Genome

- In isolation, the genome is infectious.
- Like a typical eukaryotic messenger RNA, it has a 5' cap and 3' polyadenylate tail.
- Ranges in size from 26–32 kb in length, among the largest known mature RNAs.
- Contains multiple open reading frames.
- First two-thirds of the genome contains a large 20–22 kb gene, translated via ribosomal frame-shifting into an 800 kDa polyprotein.
- The large polyprotein processes itself into 15 or 16 polypeptides that serve as a factory to replicate and transcribe the genome.
- RNA-dependent RNA polymerase and an RNA helicase are also encoded.
- There is a high rate of RNA-RNA recombination by a template-switching mechanism.

## Group-Specific Nonstructural Proteins

- Genes are interspersed among structural protein genes, in the distal portion of the genome.
- Group II hemagglutinin esterase, the only nonstructural protein with a known function, provides an extra glycoprotein on the viral surface.
- Many nonstructural proteins appear to be nonessential, not expressed, or have no role in pathogenesis.

## Coronavirus Life Cycle

- Binding to host cell receptors and fusion result in deposition of nucleocapsid and the genome into host cytoplasm.
- Host ribosome translates RNA-dependent RNA polymerase, which then makes a negative-strand copy of the genome and subgenomic mRNAs.



- mRNAs form a 3'-nested set, each with a 70–100 b leader sequence fused to an internal point on the genome, and a negative strand counterpart; these are translated into viral structural proteins.
- S, E, and M go to the ER and end up via the default secretory pathway in the ERGIC or Golgi.
- N protein and progeny genomes assemble into the nucleocapsid in the cytoplasm and then bud into the budding compartment, forming virions.
- A hundred to 1000 viruses are released via smooth-walled vesicles.

## Reverse Genetics with Coronavirus Genome

- Methods have been improved to handle large coronavirus genomes for reverse genetics but full-length clones are unstable.
- A full-length cDNA copy can be cloned into a transcription vector with a mutation of interest; RNA is transcribed from cDNA and transfected into host cells to produce mutant viruses.
- This is the only method to do reverse-genetic on the large gene 1.
- A second method uses recombination with synthetic RNAs to construct site-directed mutations that are recovered by selecting against a thermolabile deletion mutant parent virus.
- Using this second method, Masters' and Rottier's groups have created an fMHV virus from MHV with a spike protein from feline infectious peritonitis virus; this mouse virus can only grow in feline cells and recombinants pick up a restored mouse S protein with a target mutation, allowing them to grow again in mouse cells.

## Coronavirus Biology and Pathogenesis: Coronavirus Pathogenesis

Kathryn V. Holmes, Department of Microbiology, University of Colorado Health Sciences Center, Denver

### Specificity of Coronavirus Infection

- Each coronavirus infects a particular host, and much of the specificity depends on receptor interactions.
- The serogroups of coronavirus have been stable over long periods; human coronaviruses have no animal models for study.
- Spike protein and sometimes hemagglutinin protein (in Group II coronaviruses) determine the restriction of host cells.

### S protein Features

- Tip of S protein determines receptor-binding specificity.
- S2 membrane-bound domain determines membrane fusion and cell-to-cell fusion.
- Protease cleavage site between S1 and S2 is essential for infectivity and cell fusion.
- Deletions in S1 do not change receptor specificity but can change tissue tropism.
- Conformational change in S can be induced at pH 8; some regions of small intestine are alkaline.
- Antibodies to S1 and S2 are neutralizing, so these are targets for vaccine development.

### Coronavirus-Cell Interaction

- S (or sometimes hemmagglutinin esterase) interacts or maybe docks with a sugary host molecule.
- Virus then interacts with a protein-containing receptor; at 37 degrees this causes a conformational change in S that might allow interaction with a co-receptor.
- Changes in receptor lead to fusion of envelope with cell membrane and delivery of nucleocapsid into the cell.
- Coronavirus fusion domain, like gp41 and HA2, has two heptad repeats and undergoes conformational



change.

## Host Cell Receptors

- Viruses in Group I share the same receptor, aminopeptidase N, but use it in different ways; it is 150 kDa and on the surface of the enteric and respiratory tracts.
- A single glycan prevents human coronavirus from binding pig aminopeptidase N.
- All group I coronaviruses can use feline aminopeptidase N, so it may be the original receptor for all these viruses before specialization.
- Only known receptors for Group II viruses are CEACAM1 and 9-O acetylated sialic acid in mouse.
- CEACAM1A is an immunoglobulin superfamily member with a projecting region that is available on the surface of the intestine and respiratory tract; it is also a receptor for H. influenza and Neisseria.

## Jumping Hosts

- Beginning to look at how many mutations would allow a spike protein to jump hosts using targeted RNA recombination.
- Three regions conserved among all S proteins apparently are for structure, not binding.
- Looking at whether SARS virus can infect both an animal and human host, in which case there would be an animal reservoir for SARS, or whether a mutation caused the virus to jump from an animal to human host.

## Human Coronavirus Infection

- 229E has been innocuous and stable for a long time.
- Intranasal infections with 229E caused disruptions in nasal epithelium in all cases and symptoms in some.
- The same person can get the same virus repeatedly.

## Vaccine and Drug Targets

- S raises neutralizing antibody and is a good target.
- Inhibitors that block conformational change in S are good targets.
- Monoclonal antibodies that block infection may serve as a receptor blockade.
- Protease inhibitors that prevent polyprotein processing.
- Inhibiting budding, exocytosis, or secretion.

## **Coronavirus Biology and Pathogenesis: Coronavirus Transmission and Persistence**

Linda Saif, Professor, Food Animal Health Research Program, Ohio State University, Wooster

## Porcine Coronaviruses

- The porcine coronaviruses that cause enteric infections are TGEV (transmissible gastroenteric virus) and PEDV (porcine epidemic diarrhea virus).
- Porcine respiratory coronavirus (PRCV) is thought to be an S-gene deletion mutant of TGEV but they are the same serotype.
- RT-PCR and nested PCR assays can detect and differentiate between PRCV and TGEV.
- TGEV infects small intestinal villous enteric sites, occasionally the upper respiratory tract, and induces villous atrophy and a cytolytic infection, leading to vomiting and diarrhea.
- PRCV infects epithelial cells of upper and lower respiratory tract but not intestinal epithelia, so there may be a co-receptor for TGEV on those cells.
- PRRS infects cells in the lamina propria, causes moderate to subclinical respiratory disease, and repli-



cates to high titers in lung.

- The PRCV S gene retains the immunodominant epitope A, but not the C and D epitopes on TGEV.
- PRCV S gene deletions vary in size, and strains with shorter deletions may be more virulent than those with longer deletions.
- PRCV causes viremia, and virus can replicate in alveolar macrophages.
- Two point mutations in S appear responsible for going from enteric to respiratory tropism.
- For TGE, vaccine strains are intermediate between wild type and attenuated strains.
- Enteric coronavirus can be fatal in young animals, and respiratory coronavirus infections are more often fatal in adults when combined with other factors.

#### Bovine Coronaviruses

- All bovine coronaviruses belong to a single serotype and are pneumoenteric.
- Viruses infect a range of enteric and respiratory tissues.
- Have identified 42 amino acid changes at 38 sites, with 5–6 clustered changes, but no indication of what this means for phenotype.

#### Viral Shedding

- Virus is shed in feces when intestinal epithelial cells are infected and sometimes, with PRCV, when few intestinal tract cells are infected, possibly due to large amounts of virus being swallowed.

#### Animal vs. Human Respiratory Viruses

- Induce similar clinical signs and infect similar tissues to SARS, but PRCV can infect alveolar macrophages.
- Pathology and shedding periods are similar.

#### Co-Infections with Coronaviruses

- In pigs, concurrent or sequential infections of respiratory exacerbate shedding, fever, respiratory disease, and infiltration of monocytes in lung; co-infection with flu and PRCV produces enhanced respiratory disease. This may have relevance for SARS and flu outbreaks.
- Secondary bacterial infections with PRCV enhanced respiratory disease.
- Animals with lower serum antibody titers were more likely to develop bovine coronavirus infection and disease.
- Stress and commingling of animals from different locations leads to more outbreaks among cattle.
- Concurrent respiratory infections and bacteria in cows act synergistically to produce pneumonia.

#### Coronavirus Reinfections

- Reinfections with PED are symptomatic but are not with bovine CoVs or PRCV.
- Reinfections are usually mild or subclinical.

#### Crossing the Species Barrier

- Coronavirus from wild ruminants can experimentally infect young calves, indicating a potential reservoir.
- Turkey poults, but not chicks, can be infected with bovine enteric CoV, so cattle-to-bird transmission is possible.

#### Animal Coronavirus Vaccines

- The focus in animals is on passive immunity via the mother's milk, exploiting the common mucosal immune system.



- TGE vaccines induce IgA antibodies in milk.
- PRCV infections only produce partial immunity to TGE, suggesting compartmentalization in the common mucosal immune system.
- Repeated immunization at multiple sites may increase IgA titers in mother's milk.
- Attenuated vaccines for pigs, intranasal or oral, replicate poorly in intestines and produce low IgA titers in milk.
- Killed TGE vaccines given intramuscularly only induce IgG in milk and are not protective.
- It is easier to boost mucosal immune response than elicit intestinal immune response.
- Virulent TGE virus stimulates high IgA antibody secreting cell numbers and lymphoproliferative response in intestinal tract but not respiratory tract; animals are protected against diarrhea and nasal and rectal shedding.
- Virulent PRCV elicits few IgA-producing cells in intestine, but mostly IgG and lymphoproliferative response in respiratory tract; animals are partially protected against diarrhea and completely against nasal shedding.
- Systematic S vaccine given subcutaneously with incomplete foynes does not induce protective immunity to TGE, but when combined with other viral proteins, given IP, and with a potent mucosal adjuvant, it induces partial protection against TGE shedding.
- Recombinant N proteins with T-cell epitopes and S proteins may be required for maximal antibody response.
- The only existing commercial respiratory coronavirus vaccine is for infectious bronchitis (IBV) in chickens, using killed or live vaccines; effectiveness is complicated by multiple serotypes of IBV.
- Live attenuated TGE vaccine is stable over 100 passages in culture.
- An appropriate animal model is extremely important to design effective SARS vaccines

## **Coronavirus Biology and Pathogenesis: Technology in SARS Discovery**

Thomas Ksiazek, Centers for Disease Control and Prevention, Atlanta

### CDC involvement in SARS

- CDC Flu Branch became involved initially; Special Pathogens Branch became involved because of evidence of person-to-person transmission.
- Special Pathogens focused on obtaining diagnostic specimens in early days, working to identify specific diagnostic tests, the pathological process, possible agents, and tissues to focus on.
- Presence of giant cells in lung and diffuse alveolar damage suggested a viral etiology.

### Molecular Techniques

- Vero E6 cells were inoculated.
- Electron microscopy, negative stain, thin-section morphology, and morphogenesis resembled coronaviruses.
- Dean Erdman's group designed primers that can be used as a diagnostic technique on clinical materials.
- Sequence analysis suggests SARS virus is the first in a new group of coronaviruses.
- Cofactors still may be involved in causing SARS, but coronavirus is the etiologic agent.
- Antigens could not be found on giant cells from patients, but antigens usually do not persist for long in many respiratory infections.

### Diagnostic Techniques

- Vero E6 cells can be stained in an indirect fluorescent antibody (IFA) with convalescent patient serum, showing viral antigens present.



- An ELISA test was developed using antigens from infected cells.
- Western blot on convalescent patient materials show N protein predominates and is goal for recombinant protein synthesis.
- ELISA and IFA can be used on convalescent and acute sera from patients to show conversion from negative to positive.

#### SARS in Cultures and Tissues

- Virus sequences from various sites is essentially identical.
- The disease is reproduced in cynomolgus monkeys.
- Virus is identified in respiratory secretions and tissues using a number of techniques.
- Patients, including those who succumb, have neutralizing antibodies.

### Coronavirus Biology and Pathogenesis: Panel 1 Discussion

#### Tracing the Virus by Mutation

- SARS coronavirus does not have enough similarity to any known virus to identify its origin in another species.
- RNA viruses have high frequencies of mutation, but the selection of mutations is more important. SARS coronaviruses isolated have shown very few mutations and these are more likely to be epidemiological markers than biologically significant.
- We do not have the information to correlate point mutations with effects on functionality or phenotypes.

#### Stability and Reservoirs of Virus

- TGEV is stable in frozen fecal material, but is inactivated by UV radiation in summer; infections show seasonality.
- Birds and flies may serve as reservoirs of TGEV mechanically or by ingesting and shedding viruses.
- Possible fomites for SARS should be considered.
- There is some stability to the virus when dried, and it lasts for probably a few days.
- If fecal shedding occurs, it could cause sewage contamination.
- There is some evidence to suggest coronavirus can persist noninfectiously in chickens and that cats shed feline enteric CoV for months.
- Super-spreader phenomenon does not appear to be virus-specific.

#### Immune Response

- There is no correlation seen in Hong Kong between mortality and seroconversion, as seen with IFA.
- PRCV is shown to elicit high levels of alpha-interferon.
- NK cells play a role in TGEV infection, and there is evidence of antibody-dependent enhancement of immune response.

#### Virus Detection and Culture

- SARS coronavirus has been grown in Vero, Vero E6, LLCMK2, primary monkey kidney, and PK15 cell lines.
- PCR shows RNA copies in stool, sometimes at higher levels than respiratory secretions.
- In some cases SARS virus has been amplified from plasma but not consistently.
- In animals, peak viremia usually correlates with peak shedding; correlates are better with ELISA for viral antigen because PCR methods detect virus before symptoms appear.



## Exacerbating Infection

- In feline models, certain types of vaccine-introduced antibodies can enhance infection, primarily including monoclonals against epitopes of the spike protein; one epitope did not
- Factors could be involved in exacerbating respiratory infections.

## Session II: On the Front Lines

Moderated by Scott Hammer

### On the Front Lines: Clinical Spectrum of SARS Infection

Larry Anderson, Centers for Disease Control and Prevention, Atlanta

#### Early SARS Transmission

- Index Case was a visitor to Hong Kong from China; ten people were exposed and transmitted SARS to other countries.
- SARS is predominantly transmitted in hospital settings.
- Close contact is the predominant mode of transmission in droplets, fomites, direct contact, and autoinoculation. Some airborne transmission may have occurred.
- Infection control practices limit transmission, even from super-spreaders.
- Transmission was controlled or stopped in Canada, Singapore, Thailand, and the United States.
- Because of success with control, patients likely transmit only when sick.

#### Symptoms and Illness

- Symptoms begin with fever, then cough, shortness of breath, interstitial pneumonia on X-ray, and lymphopenia.
- Illness is generally more severe with older patients.
- There is a high rate of severe illness with SARS.
- Case fatality rate is going up, mostly likely due to ways of accounting for cases.

#### Diagnosis and Detection Issues

- We do not yet know what the best specimen is at certain times of illness for detecting infection.
- Diagnosis to date has used electronmicroscopy, isolation of virus, detection of antigens in tissue, and detection of viral RNA by PCR.
- Virus has been detected in respiratory secretions, stool specimens, urine specimens, bronchial lavage specimens, and in lung and kidney tissue.
- Antibody has not been detected in non-SARS patients.
- SARS in immune-suppressed patients could be a problem in the future.

### On the Front Lines: Clinical Experience in Toronto

Donald Low, Mt. Sinai Hospital, Toronto

#### First Toronto SARS Cases

- Toronto's Index Case stayed at the Hotel Metropole in Hong Kong on February 21, returned to Toronto February 23, died at home on March 5.



- Index Case's son became ill, went to hospital March 7, and died on March 13.
- The rest of the family was brought in for isolation on March 13.
- Case B was admitted without protection and was the first sign that spread outside families had occurred, primarily among hospital workers and visitors.
- Case B's wife became ill, indicating a need to remember the family members before they can spread the virus.
- Case C was misdiagnosed early with congestive heart failure, causing spread to cardiac health care workers.
- The close-knit BLD community was the only case of community outbreak in Toronto.

## Clinical Features and Outcomes from 144 Toronto Patients

- Among the first 144 patients at Toronto's Mt. Sinai hospital, females were prevalent, as are health-care workers (51 percent), and median age is 45.
- Clinical symptoms begin with aches and pains and fever within three to ten days of known exposure. Diarrhea and cough follow.
- Patients under home isolation often left the home, causing new infections.
- Emergency departments, doctors' offices, and occasionally hospitals recognize the disease most often.
- Median time from visit to admission is three days, so many people were sent home.
- Chest X-rays are normal in 25 percent of early patients, pneumothorax occurred in 4 out of 144 patients, and about 30 percent showed infiltrates in both lung fields.
- LDH is high in 90 percent of patients, perhaps reflecting lung disease, and creatine kinase was abnormal in 40 percent of patients.
- Ribavirin and steroids need to be evaluated in clinical trials for effectiveness; in a small sample, they did not appear to affect viral load.
- Artus RT-PCR system was used to detect virus in lungs from autopsy and all 11 samples taken were positive.
- Duration of illness before death was 20 days; those who died sooner were more likely to have high viral load.
- Mortality rate is going up, likely because of the denominator used in determining probable cases.

## Infection Control

- Contact and droplet spread is the most likely mode of transmission, and possibly occasionally airborne contact.
- Precautions used include a N95 face shield, hairnet, gloves, gowns, and hand-washing.
- The order of removal of protective gear is important.

## On the Front Lines: SARS: An Update from China

Chen Zhu, Shanghai Second Medical University, People's Republic of China. Delivered by Dr. Scott Hammer

## Impact on China

- China is hardest hit by SARS, with 5000 cases, almost half in Beijing.
- China has devoted a science and technology task group to work on SARS epidemiology, diagnostics, treatment, disease mechanism, biosafety and infection control, and education.

## Task-Force Activities

- Modeling and predicting the trajectory of the epidemic.



- Screening possible drugs and vaccines, including some from traditional Chinese medicines.
- Screening humanized monoclonal antibodies.
- Looking into antisense oligonucleotides or RNA interference
- Determining host susceptibility issues.
- Building collaborative scientific relationships and sharing resources.

## On the Front Lines: Panel 2 Discussion

### Assessing Actual Cases and Mortality Rate

- Patients were admitted with flu-like illness and never developed pneumonia; a serological test is needed.
- If suspect SARS cases are excluded from accounting, the mortality rate jumps from 7 to 15 percent.
- We do not yet know what an asymptomatic SARS infection looks like or how frequently it occurs; this also affects the mortality rate.
- The concept of super-spreaders is confounded by infection control; some so-called super-spreaders may be shedding more virus or may be more efficient transmitters.
- Case reporting is further confounded because gastrointestinal and respiratory illness are commonly reported by people returning from travel.

### Sequelae with SARS

- A connection between SARS and diabetes has been observed in a number of countries but more data is needed.
- Psychological impacts of fear among recovered patients and communities will need to be addressed.
- Recovered patients are anecdotally reporting low energy, hyperactive airways, and cough.

### Site and Extent of Infection

- Initial infection appears to be in the upper respiratory tract but the mode of spread to other tissues is not known.
- Patients appeared viremic but this may have been a result of cytokines.
- In Hong Kong, virus is found in epithelial sheds, though it does not appear to cause CPE there.
- Viral load goes up in the first week, following onset of symptoms, and decreases thereafter.
- There is a six-log range of viral load over the course of illness, possibly explaining super-spreading.

## Session III: Approaches to Vaccines and Drug Development

Moderated by Scott Hammer

### Approaches to Vaccines and Drug Development: Blocking SARS Virus Fusion

David Ho, Aaron Diamond AIDS Research Center, New York

#### Visit to China

- The concern among people and the socioeconomic impact are high in China.
- China recognizes that it missed opportunities to address SARS more effectively and is now galvanized against it.
- Efforts include sequencing to enable molecular epidemiological testing, vaccine research, searching for



an animal source, and building BL3 and BL4 labs.

S Gene Structure Resembles HIV gp 41

- Spike protein has two regions of alpha-helical structure with heptad repeats, like gp41 and other envelope virus structural proteins.
- Binding of gp120 to target host cells results in conformational changes that result in a hairpin formation, close approximation of viral and host membrane, and fusion.
- Peptides derived from repeat regions can inhibit virus entry and replication of HIV and other viruses; T-20, or Fuzeon, has recently been licensed as a drug.
- Evidence suggests that peptides lock the virus into an intermediate conformation, preventing fusion.

Testing peptides against SARS Coronavirus

- A qualitative CPE assay and a plaque assay in Vero cells have been used to test twelve peptides; five showed varying degrees of antiviral activity.
- Peptide therapeutics are difficult to make and are likely to be injectible rather than oral pills; development is rapid and they are generally not very toxic.
- Activity may be improved upon by optimizing the peptide sequence used.

## **Approaches to Vaccines and Drug Development: Lessons in Interventions for SARS**

Frederick Hayden, University of Virginia School of Medicine, Charlottesville

Current Vaccines and Antivirals in Humans

- Inactivated vaccines for flu induce strain-specific durable immunity and antibody to hemmagglutinin correlates with protection, but the virus changes rapidly.
- Natural infections induce incomplete protection against RSV and human CoVs, so repeat infections occur.
- Monoclonals to RSV fusion protein effective against lower respiratory tract disease in high-risk infants.
- Formalin-inactivated whole virus vaccine against RSV-induced aberrant immune responses and worse outcomes in infants in the 1960s.
- There is no proven vaccine or antiviral for human respiratory CoVs.
- Effective application of these modalities is important.

Flu Antivirals

- Development of neuraminidase inhibitors has been rapid when linked to virologic and clinical surveillance, access to populations, and accurate diagnosis.
- Antivirals may be an adjunct to or substitute for vaccine, offering several strategies that may be applicable to SARS.
- Oseltamivir reduces risk of hospitalization by half.
- Using treatment of an index case and close contacts with neuraminidase inhibitors, no resistance or transmission occurred.

Current Drugs used for SARS

- The sequence of viral load from low to peak at day 10 and subsequent decline offers a window of opportunity for antiviral intervention.
- Corticosteroids may have contributed to protracted and increased viral replication.
- Dexamethasone causes a delay in clearance of respiratory syncytial virus in infants.



- Steroids enhance replication and mortality in mice with pneumonia.
- Intranasal steroids delay viral clearance in rhinovirus in adults and increase the risk of acute otitis media in children.
- In vitro assays show no inhibition of SARS coronavirus replication by ribavirin.

#### Interferon protection for CoVs

- Alpha and beta interferon have activity against SARS CoV when screened in vitro.
- Intranasal interferon at a relatively high dose (common cold unit) shows 55 percent reduction of infection and 85 percent reduction of development of colds.
- Lower doses of intranasal interferon did not protect against infection but moderated the frequency of colds.
- Need to understand whether there are over-exuberant or deficient responses in SARS that may be supplemented.
- Controlled clinical trials will be essential.

#### **Approaches to Vaccines and Drug Development: Status of Drug Screening vs. SARS**

Catherine Laughlin, National Institute of Allergy and Infectious Diseases, Bethesda

#### Collaborative Screening of Drugs

- CDC, USAMRIID, and NIAID have are exploiting an existing collaboration to screen drugs against the SARS pathogen.
- FDA is following the screening to be prepared to act quickly on any potential drugs.
- To protect intellectual property of sponsors providing agents for testing, a single material transfer agreement / CRADA is being used.
- Jack Secrist at Southern Research Institute contacts sponsors, acquires compounds, puts compounds under code for testing, and returns data to sponsors.

#### Screening Assay

- An in vitro CPE-based assay is based on Neutral Red uptake, measuring living cells.
- Capacity for testing is 400 compounds per week.
- Follow-up assays are more intensive for plaque or yield reduction.
- Testing is done on Vero E6 cells in 96-well plates, with four drugs and five dilutions per plate, covering 625 dilution-range concentrations from 1 to 100 micrograms per milliliter.
- Fifty percent inhibitory concentration is measured.
- The window of killing virus without killing cells is the selective index; ten or better is preferred but initial screening looks for anything over two.
- One hundred and twenty compounds have been screened; those with activity have been beta-interferon, rimantadine, and cysteine protease inhibitors.

#### Possible Viral Therapeutic Targets

- Cysteine protease inhibitors.
- RNA-dependent RNA polymerase.
- Helicase activity.
- Other targets in genome replication and transcription.
- Assemblyosome.
- N protein.
- Fusion between virus and host, or cell-to cell fusion.



Testing Priorities (beginning with highest)

- FDA-approved antiviral drugs.
- Antiviral drugs in clinical development for other indications.
- FDA-approved drugs for anything that also work against SARS.

## **Approaches to Vaccines and Drug Development: Adenovirus Vector Technologies for Vaccines**

C. Richter King, GenVec Inc., Gaithersburg, VA

Advantages of Adenovirus Vectors

- Can serve as a vaccine platform for several types of disease.
- Trigger strong and potentially medically significant immune response via a variety of different cellular pathways.
- Are well tested in clinic, and are well-tolerated as a vector system and vaccine system.
- Methods to bring AdVectors into the clinic are well established, and manufacturing and quality control procedures are in place.
- Used as an immunization boost in animals, immune response increases greatly, measured by antibody titer and cell-mediated response.
- High numbers of homologous vector can be produced and purified quickly.

Manipulating AdVectors

- All AdVectors are missing essential genes, so they can no longer replicate, making them relatively safe, and freeing space for addition of many expression cassettes.
- Vectors can be optimized for performance and can be used to quickly test many conformations of antigen or multiple antigens.
- DNA can be flexibly moved around using homologous recombination.
- Libraries of genes or various modifications of a single gene can be used to produce a library of AdVectors for testing.
- Broadening the tropism of AdVector can improve antigen presentation by almost an order of magnitude and improved cellular response to antigen.

## **Approaches to Vaccines and Drug Development: Some Approaches to Vaccine Development**

Thomas Monath, Acambis, Cambridge, MA

Considerations for a Potential SARS Vaccine

- Pursuing a vaccine against SARS will require multiple approaches, a significant effort, and will require collaboration among molecular biology, virology, immunology, animal experimentation.
- A vaccine needs to be administered pre-exposure, induce protection against clinical disease and perhaps against infection and transmission, be given in few doses, elicit neutralizing antibody response, be safe and immunogenic, and be manufactured efficiently on a large scale.
- Robust mucosal immune response may be needed and is difficult to achieve with many vaccines.
- Antigenic variation of SARS CoV needs to be monitored.

Animal CoV Vaccine Results

- Mucosal immunization strategies are required to protect newborn animals.
- Partial protection is demonstrated with live or inactivated vaccines in some cases, but is not durable.



- Subunit vaccines are not particularly effective except for priming and boosting.
- Antigenic variation is a problem with IBV.
- Live vectors show promise in some models.
- Feline infectious peritonitis vaccine enhances disease.

#### Evaluating Possible Approaches

- For inactivated whole-virus vaccine, the method of inactivation may be important to preserve the native structure; these may not provide long-lasting immunity and require multiple boosting.
- Recombinant subunit vaccine will require adjuvant and likely mucosal delivery.
- Live vectors or replicons may be problematic because of anti-vector immunity.
- Live-attenuated virus may be the best approach to induce mucosal and systemic immunity, or could be part of a priming and boosting regime, and would not have manufacturing issues.
- DNA alone has not been a successful vaccine and faces regulatory issues.

#### Timeline and Cost

- An aggressive timeline puts a "go/no-go decision" at 18 months after beginning vaccine development.
- Development would cost 60 to 100 million dollars and five to six years at a minimum.

#### **Approaches to Vaccines and Drug Development: Panel 3 Discussion, including Richard Colonna (Bristol-Myers Squibb), Michael Dunne (Pfizer) and Emilio Emini (Merck)**

##### Potential of Drug and Vaccine Success

- SARS CoV has good potential for vaccine development because it grows well in cell culture at high titers, there is a plaque assay, it can be titered, there are good targets, assays are relatively quick, and RNA alone is infectious.
- Likely successful drug or vaccine targets are those involved in entry, protease, and RNA polymerase.
- Induction of neutralizing antibody ought to be the first objective in vaccine development, before worrying about inducing mucosal immunity.

##### Sharing Information

- There is a lot of information about immune response and protective responses in animals using the MHV system, and some may be directly translated to SARS.
- Some collaboration will go on amongst pharmaceuticals and is already occurring between academic or government scientists and pharmaceutical or biotech scientists.

## **Session IV: Future Perspectives on Emerging Infections**

Moderated by Allan Rosenfield

### **Future Perspectives on Emerging Infections: Special Pathogens in Three Cultures**

C.J. Peters, University of Texas Medical Branch, Galveston

#### Emerging Disease Factors

- Exploration and now travel cause mixing of viruses around the world, most notably with Yellow Fever and West Nile Virus.



- West Nile emergence may be understood by considering that it is a phylogenetically-related virus that uses a mosquito vector that hides in toilets of airplanes.
- Many viruses have evolved during and after human urbanization, such as measles and smallpox.
- Factors contributing to emergence have gotten worse in the last ten years, as assessed by an Institute of Medicine report.
- Evolutionary opportunities for viruses generally drive adaptation.

## Expected Trajectory for SARS

- Generally with emerging diseases, an individual doctor makes a diagnosis, CDC tracks it through local and state health departments, NIH builds research and knowledge through academia, and industry makes products to fill needs.
- We need to get ahead of this process to beat SARS in a timely and effective way.
- Seasonality, healthcare budgets, and local cultures, particularly in Third World countries, will make eradication difficult.
- Strict U.S. standards may require that drugs or vaccines developed here be manufactured and produced overseas.
- There needs to be a group nationally responsible for antiviral or antiinfective drugs and vaccines.

## Future Perspectives on Emerging Infections: SARS and Public Health Systems

Marcelle Layton, NY City Department of Health

### Preparedness in New York

- Thirty thousand international travelers arrive at a New York airport every day; we must be aware of diseases that may not receive much attention in medical school.
- New York City has accelerated emergency-preparedness since the West Nile virus outbreak and now takes an all-hazards approach.
- Plans are being practiced for mass prevention and emergency communications.

### Surveillance Systems

- Traditional surveillance depends on medical and lab communities to recognize something unusual and reporting it; the medical examiner's office reports any unexplained potentially-infectious deaths.
- Syndromic surveillance detects an increase in prodromal symptoms to try to detect outbreaks earlier, recognizing that people may go to a pharmacy or hotline before a doctor or hospital.
- A number of surveillance systems more reliably screens out artifacts; when many systems respond there is more cause for concern..

### Communication

- A broadcast alert system is used to communicate with hospitals, keeping clinicians updated on what, when, and how to report and good triage protocols.
- Communicating uncertainties and handling the demand for information is important.

### Contingency Planning

- There is partnership with other agencies to coordinate responses; legal, security, and logistical issues are being addressed to plan for an isolation and quarantine situation.
- Search capacity has been increased to mobilize response via hotlines, databases, lab tests and analysis, and teams to review charts and interview patients.
- Hospitals are being made to think through dealing with a surge of contagious patients, communication



procedures, and training staff.

- Proposed changes to the NYC Health Code will strengthen the ability to do detention, isolation and quarantine.
- SARS teams are in place, doing active case and contact management; all staff are fit-tested to be able to do home visits.

### **Future Perspectives on Emerging Infections: A View from the Field and the Bridge**

John La Montagne, National Institute of Allergy and Infectious Diseases, Bethesda

- SARS is an important problem because it is unpredictable and is causing dramatic impacts and disruption.
- Collaboration and cooperation amongst agencies, between the public and private sectors, and internationally are key to addressing SARS.
- NIAID is convening a meeting on May 30 to talk about research activities related to SARS; information is available at <http://sars.iqsolutions.com>.
- NIAID has initiated grant and contracts programs for diagnostic, vaccine, and therapeutic development against SARS.

### **Future Perspectives on Emerging Infections: Panel 4 Discussion**

- A good sample set would have helped at the beginning of the outbreak, so that different labs would have acute and convalescent samples from the same patients.
- SARS may not be a disease of animals; in fact, the best animal reservoir vector is one that does not show signs of clinical disease.