SUPPLEMENTAL HANDOUT:

This lecture is one of two on respiratory viruses. Many viruses cause respiratory disease, but the prominent ones are orthomyxoviruses (influenza) and paramyxoviruses (respiratory syncytial virus and parainfluenza virus). This lecture has some background, and then some details on the orthomyxo (influenza) viruses. The other lecture is primarily about the paramyxoviruses and is provided by Dr. Baker.

*By the end of the lecture, you will be sufficiently familiar with influenza virus biology, epidemiology, and medical prevention measures, such that you can communicate expertly on this topic with your patients and colleagues.*

Slide 2:

Our general familiarity with influenza – induced respiratory disease may cause us to take the condition lightly, but it should be noted that influenza can cause tremendous mortality and morbidity, as it did in 1918. US life expectancy dropped precipitously in 1918 due largely to lethal influenza infections in young people.

So-called “excess deaths” were close to a million in the USA in 1918; this corresponds to 2% of infected individuals proceeding to death. Remarkable and disturbing. Subsequent influenza pandemics have been less severe but the excess deaths are still quite high.

If you want to know more about 1918 influenza and influenza pandemics in general, you can read these “lay audience” – type articles from Smithsonian:

How the horrific 1918 flu spread across America
http://www.smithsonianmag.com/history/journal-plague-year-180965222/#sCRoXpgCTGR6ZU5H.03

Medical researchers are racing to create a revolutionary flu vaccine
http://www.smithsonianmag.com/science-nature/stop-lethal-virus-180965217/#96ROVvC9gwEFQODw.03

Is China ground zero for a future pandemic? Hundreds there have already died
http://www.smithsonianmag.com/science-nature/china-ground-zero-future-pandemic-180965213/#CAUz0Gjwhdfhe2jT.03

Slide 3:

Transmission:

Key fact: Most viruses take inhalation route.
Aerosol droplets can contain viruses. Transmission is often inefficient. Host lung has mucociliary motor, and alveolar macrophages as defense.

Exceptions to low transmissibility are seen in pandemics. Influenza pandemics show the virus is highly transmissible. 15-40% attack rate (percent of population infected) in a typical pandemic.

**Slide 4:**

Once virus is contracted, replication is rapid. Note the short incubation period for flu. Note that one infectious cycle is completed in 10h. 1 virus to 1,000 per cell in 10h, then 1,000 to 1,000,000 in next 10h, and so on.

There is a 1-3 day incubation period, then disease

Note the infectious period matches the symptom period, although there can be shedding prior to symptoms. Some individuals (about a third) get infected but do not have disease. These are high transmitters of virus.

**Slide 5:**

Symptoms of influenza virus. Fever, chills, cough, congestion, aches, diarrhea (which is secondary to lung injury and does **not** come from influenza infection in the GI tract). The infection resolves in 5-7 days.

Note that symptoms are not specifically diagnostic of influenza. Other infections can cause similar symptoms.

**Slide 6:**

The rare but severe respiratory viral disease is acute respiratory distress syndrome (ARDS). This can come from influenza A virus (IAV) and some other resp viruses.

Note the acute lung consolidation. This is immunopathology, it is essentially an overexuberant innate immune response to the infection. There is edema in lungs. Mechanical ventilation may be necessary for survival.

**Slide 7:**

Pathology of ARDS includes neutrophil and lymphocyte invasion, edema, hyaline membranes.
Slide 8:

Most complicated slide here. This is what your patients experience after getting productively infected with influenza.

Virus infects epithelia. Type I IFNs produced and secreted.

NK cells activated. These control infection by killing infected cells. Lung gets damaged somewhat.

Dendritic cells (DC) take up viral antigen. The traffic to lymph nodes. They activate CD4 T cells. CD4 T cells control adaptive immune responses by secreting cytokines. Cytokines activate macrophages. Macrophages kill viruses and virus-infected cells. There is some lung damage.

CD4 cells and DC activate CD8 CTL. CTL kill virus-infected cells. There is some lung damage.

CD4 cells instruct B cells to make antiviral antibodies. There is an anamnestic response. Long-term immunologic memory.

Overexuberant cytokine levels cause ARDS.

Slide 9:

Symptoms in severe cases; comorbidities and susceptible patient populations: This is important clinically. The susceptible populations are the best candidates for antiviral therapies.

INFLUENZA VIRUSES:

The focus is on influenza type A, because about 70% of human influenza diseases are caused by type A. There are also influenza types B and C, which cause the remaining 30% of flu disease.

Slide 11:

Influenza is enveloped minus-strand segmented RNA virus, pleiomorphic structure, 8 single-stranded minus-strand RNA strands in each particle.

A typical USMLE step 1 question asks whether an organic solvent might kill a certain virus. You need to know whether the virus is enveloped (will be killed by organic solvent) or nonenveloped (will not be killed). IAV would be killed.

Slide 12: IAV structure
These particles are enveloped, as such, they are delicate and easily destroyed by common hand soaps. The inside of the particles harbor RNA strands. The outside of the particles are studded with glycoprotein projections.

In a highly schematized form, the influenza virion can be seen to have 8 RNA strands. The influenza virus genome is segmented. All 8 RNA strands must be introduced into a cell to establish an infection. The 8 RNAs are covered up with N (nucleoprotein) proteins and are surrounded by a lipid envelope. Also associated with the RNAs are PB1, PB2, PA. These are RNA polymerase enzymes that are needed to start viral transcription upon infection. The envelope gets some rigidity by a protein called M1 (matrix). There are holes in the envelope (pores) that are made of an ion channel called M2. The glycoprotein projections include HA and NA, hemagglutinin and neuraminidase, respectively.

Slide 13:

Don’t be alarmed by this slide. It is less complicated than it appears.

There are three key points:

1. Virus enters cells apically (one virus enters) and newly-made progeny viruses (1,000 or more progeny) egress after 10h, apically. So the virus stays in the respiratory tract. Systemic influenza is very rare.
2. Influenza uses the host cell nucleus for its replication. Most RNA viruses replicate entirely in the cytoplasm but flu needs the nucleus. The viral RNAs enter into the nucleus where they are transcribed to make mRNAs. The mRNAs then go out to the cytoplasm where they are translated to make new viral proteins. These viral proteins include polymerases, which return to the nucleus to amplify infection, and they include HA, NA, M2, which go to the plasma membrane to form platforms for new virus budding. The RNAs are also replicated, to make antigeomic copies, which are then copied back to more genomic RNAs. These components congregate at budding sites near the plasma membrane and new viruses are budded off.
3. Not on this slide, but relevant, is that influenza is an aggressive directly cytolytic virus. Many epithelial lung cells die as a result of direct virus infection. Cell debris are found in lung airways, contributing to disease. The virus also encodes proteins that resist host responses to infection, so the virus can continue aggressively for days, producing high yields of progeny, even in the face of some host antiviral cytokines.

Slide 15:

With the understanding of the influenza virus and its replication strategy, we can now discuss how the virus causes epidemics and pandemics. The question of how epidemic and pandemic
strains arise is explained molecularly. We know now about HA and its structure and the place where protective anti-HA antibodies bind (this slide).

**Slide 16:**

So it is straightforward to appreciate that these antibody sites can mutate. Flu is an RNA virus, highly mutable, spawning mutants that may be altered in HA epitopes, and thus somewhat resistant to anti-HA antibodies, making them able to expand in humans and cause epidemics. Key concept here is that point mutations, usually in regions encoding HA antibody epitopes, can change virus structure and antigenicity leading to viruses that may cause epidemics. This is referred as “genetic drift”.

**Slide 17:**

Genetic drift explains why we have to re-vaccinate yearly. This slide is from NIH / NIAID.

**Slide 18:**

Pandemic strains are more extensively altered. They come from RNA segment reassortments. Remember that flu is widespread and each particle has 8 RNA segments.

**Slide 19 and 20:**

So it is possible for two flu types to infect a single cell, in this example, a single duck cell, which then puts two sets of 8 RNAs into the same cell. If these RNAs can freely reassort, then there are $2^{\exp8} = 256$ combinations of output viruses. Some may be uniquely pathogenic and resistant to currently circulating antibodies. For example, the one depicted here has bird RNAs 4 and 6 and therefore would have human virus core components but bird virus glycoprotein projections. This may be a novel pathogenic reassortant that can cause a pandemic. Key concept here is that RNA segment reassortment can change virus structure, antigenicity and function dramatically, leading to reassortant viruses that may cause pandemics. This is referred as “genetic shift”.

**Slide 21:**

One can appreciate the ever changing influenza viruses with this chart of influenza A strains in the human population. Note type A/H1N1, common in the 1930s and 40s, then disappearing in the 60s and 70s, presumably because the human population was largely immune. Then the same strain reappeared in the 80s and 90s. The susceptible hosts in the 90s may have been yourselves, as you could not have acquired resistance to this strain from your parents or grandparents (antibodies are not inherited).
There is the question of where the A/H1N1 was located during the 60s and 70s. The best answer is that it was circulating in birds and perhaps other animals at that time, having little opportunity to invade humans until susceptible human hosts become more abundant.

Slide 23:

IAV needs to be controlled through vaccination. It takes many millions of eggs to produce enough flu vaccine for mass vaccination. Candidate vaccine strains are grown in eggs, then purified (at least partially purified), inactivated with crosslinking chemicals, then injected intramuscularly.

Patients with egg allergies may react poorly to injected flu vaccines.

It takes about a month for antibodies to be elicited. That is why vaccination efforts take place in the fall.

There are also live-attenuated influenza viruses that can be administered intranasally, and these develop IgA antibodies.

Slide 24:

The protection from flu comes with antibodies. The antibodies elicited by the vaccine will bind to HA proteins. This prevents virus from infecting host cells.

A typical USMLE step 1 question asks about viral vaccines. What step in the virus infection process is blocked by the vaccinated individual? Answer is that virus – cell entry is blocked – blocked by antiviral antibodies.

Slide 25:

A key reason why influenza viruses are classified into types and strains is because individual strains are the starting material for vaccines. Also, influenza viruses change so much through mutation and reassortment that vaccines have to be reformulated yearly, so that they represent the currently circulating strains. Classification is based on broad serotype (there are three, A, B and C; A is the most clinically relevant), host origin (remember, influenza is a bird and animal virus, so there are many hosts), the place and year isolated, and the HA and NA subtypes. The HA and NA proteins are the principal immunogens in the vaccines.

Last year’s vaccine formulation contained three strains, two type A and one B. As you may know, last year’s vaccine formulation slightly missed its mark. The viruses causing disease last year were not exactly the same as these vaccine strains.
You might be asking how a vaccine injected intramuscularly could protect against a virus that enters and infects the respiratory tract. It turns out that circulating IgG can escape into the lung airway spaces, the mucus layers, and anti-HA antibodies can bind HA. The way that the antibodies protect against infection is as one would expect. They antibodies stick to the HA and prevent HA from binding cells, and/or prevent HA from catalyzing virus-cell membrane fusion. The higher the affinity of the antibodies for critical parts of HA, the more effective they are at protecting the individual from infection.

By the way, for those of you interested in vaccines (future pediatricians), you may want to review the recommended vaccination schedules, which include influenza virus vaccines, at http://www.cdc.gov/vaccines/schedules/downloads/child/0-18yrs-child-combined-schedule.pdf

**Slide 27:**

For those who cannot tolerate vaccines (immunocompromised, allergic to eggs, unable to elicit protective Ig), antiviral drugs are the appropriate therapeutic route. This is especially warranted if disease is severe and influenza virus is identified in the laboratory. To identify influenza, there are at least four methods; each way has advantages and disadvantages.

**Slide 28:**

There are two types of anti-influenza drugs, the amantadines, which target M2, and the NA inhibitors, which target NA.

**Slide 29:**

The amantadines prevent an ion channel called M2 from functioning. This prevents the interior of the flu virus particle from becoming acidified in the endosome during entry. Acids (labeled as H+ in the figure) must enter the virion interior to loosen up the contacts between RNAs and M1. If this does not happen, then the RNAs (labeled as RNPs in the figure) will not flow into cytoplasm and there will not be any infection.

Unfortunately, much of the circulating influenza A is already amantadine resistant. So these drugs are losing their clinical effectiveness.

**Slide 30:**

The other class are neuraminidase inhibitors. Neuraminidase is a “receptor destroying enzyme”. It strips away sialic acids from infected cells, so that when new viruses bud off of the infected cells, they will not re-bind back onto the same cell from which they arose. If the NA is blocked by the inhibitors, then the viruses will re-bind, and this restricts the infections to a very
localized area, effectively preventing the expansion of infection. This brings considerable clinical benefits, reducing disease times about in half.

The NA is a tetramer, sticks out from virions, has sites for binding and then cleaving away sialic acids. The inhibitors are competitive, they bind into the active sites (in red here), blocking NA enzyme action.

**Slide 31:**

Something to remember is that the start of an influenza infection can be understood by focusing on the HA and NA proteins. These are the communicating external parts of the virus. The virus gets into the lungs and it sticks to lung cells via the HA.

The blue projections are HA proteins, some of which are sticking to glycans that extend from the lung cell. In the magnified inset at right, it is shown that sialic acid sticks into the HA. HA is a lectin (a protein that binds a sugar)

Sialic acid is common on lung epithelia of mammals and birds. IAV infects mammals and birds. IAV is primarily a bird virus.

**Slide 32:**

This slide depicts virus budding, then release from cells after NA cleaves away the sialic acid receptors. There is no such release in the presence of neuraminidase inhibitors (bottom panel), because the virus sticks back onto sialic acids, via its HA proteins.

**Slide 33:**

It is valuable to expand here at the end, beyond influenza, to emphasize that there are several respiratory viruses, including:  
(some of these are covered in other lectures)
Rhino
Corona
Adeno
Influenza
Para-flu and RSV
HSV and EBV

**Slide 34:**

Different resp viruses:
Key fact: Same virus can cause different disease patterns (bronchiolitis, croup, pneumonia) depending on extent of infection and host response.

**Slide 35:**

The rhinoviruses are small nonenveloped plus-strand RNA picornaviruses. The central point here is that rhinoviruses are extraordinarily diverse. There are well over 100 serotypes. Therefore, vaccines against the common cold virus are not likely (too many serotypes would have to be included in the formulations). These viruses account for about 30% of common colds.

Adenoviruses also include ~ 50 serotypes. These cause mild respiratory infections, and also “pink eye”. There are some vaccines to some Ad serotypes and they work well. Most individuals have circulating Ad antibodies.

Coronaviruses cause 20-30% of common colds and are also a causative agent of croup. Some rare strains such as SARS and MERS are highly virulent, causing lethal respiratory distress, often termed Acute Respiratory Distress Syndrome (ARDS) and subsequently, Acute Lung Injury (ALI). The ARDS is frequently caused by overexuberant cytokine release, the ALI can ultimately give rise to fibrotic lung tissue and permanent pulmonary dysfunction.

**Slide 36:**

In my opinion, this USMLE Q bank question is very difficult, but I put it out here for you because it can demonstrate how even a tough question can be answered by a process of elimination. Small changes in influenza virus surface glycosylation cannot be considered genetic shift or drift, as these terms have precise meanings in influenza virology, with shift involving gene reassortment and drift involving point mutations (slides 24 and 26). Therefore, choices A, B, D and E are eliminated. It is possible to get to the “C” answer without knowing about camouflage. We did not discuss camouflage in the lecture – it is when N-glycans cover up an antibody-binding site, making it so that the antibodies cannot bind viruses or block infection.