SLIDE 1 Vaccines & Evasion Strategies. Vaccines are perhaps the greatest achievement of public health. Vaccines have significantly reduced morbidity and mortality worldwide. However, access to care and immunizations are not uniformly available around the world, so millions of deaths occur each year despite the availability of cheap vaccines. This presentation will discuss the application of the humoral immune response and antibodies to create vaccines. In addition, some microbes do not make it easy for the immune system to destroy them – they play defense! Some organisms use the immune system to their advantage, where they can hide inside immune cells, or use antibodies to increase their uptake into certain cells. Many organisms have developed methods to change their appearance & avoid antibodies.

SLIDE 2 Learning objectives for Week 8: Vaccines & Evasion Strategies

- Identify and explain the role of vaccines in the production of immunity
- Identify particular vaccine types
- Identify the mechanisms by which vaccine stimulate immune cells
- Identify the mechanisms by which vaccines stimulate long-term immunity
- Identify the immune evasion strategies of selected microorganisms

SLIDE 3 A live, virulent organism cannot be used as a vaccine because it would cause the very disease it should prevent. Consequently, the first step in making a vaccine is to separate the two effects of disease causing organisms. In practice, this means isolating or creating an organism, or part of one, that is unable to cause full blown disease, but that still retains the antigens that will induce the host's immune response. Immunizations are neither 100% safe nor 100% effective. For example, some vaccines contain thimerosal (a mercury containing compound), as a preservative, since the 1930s. Because of possible links of this compound to neurotoxic problems (& autism also of concern), the use of thimerosal in most vaccines has been discontinued. Another example is the live polio vaccine that has caused several cases of paralysis because the attenuated virus in the vaccine reverted to the wild type and resulted in serious disease. Currently, the United States recommends giving...
doses of the killed polio vaccine to build immunity before vaccination with the live attenuated strain to prevent complications. The combination of these 2 vaccines has eradicated polio from the United States. Every person’s immune system is unique. Consequently, some people may not generate protective immunity to a disease even though they have been vaccinated. This is a common concern with the Hepatitis B vaccine, and many health professionals are tested to see if the vaccine worked (blood test for Hep B antibodies) before working in a hospital. Another example is the smallpox vaccine. The last outbreak of smallpox in the United States occurred in NYC because of a man that had been vaccinated for smallpox but the vaccine did not "take". Consequently, he was not protected & was infected in Mexico (where many cases of smallpox still occurred) before traveling to NYC. Once in NYC, he became ill and was admitted to a hospital. He was mis-diagnosed and the disease spread to health care workers & other patients in the hospital. His death and the outbreak of the disease was a public health emergency in NYC that resulted in a mass vaccination campaign. Over 6 million people were vaccinated in 3 weeks to prevent the spread of the disease.

SLIDE 4 Vaccines are very effective in preventing disease not only in individuals, but also in communities. This type of protection is called "herd immunity." When a disease spreads from one human to another, it requires both an infected person to spread it and a susceptible person to catch it. Herd immunity works by decreasing the numbers of susceptible people that can catch the disease. Once the number of susceptible people drops low enough, the disease will disappear from the community because there are not enough people to carry on the catch- and-infect cycle. The greater the proportion of vaccinated members of the community, the more rapidly the disease will disappear. This is the reason that children are often
required to be vaccinated before attending school. It is thought that at least 80% of the people in the community must be protected by a vaccine to achieve herd immunity. But, herd immunity offers no protection for some diseases, like tetanus, which is not contagious. It is also important to keep in mind that a few people may not be protected from the disease despite being vaccinated. About 1 or 2 of every 20 people immunized will not generate an adequate immune response to a vaccine.

<table>
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| **Why Needles??**  
• Plasma cells move to the bone marrow, after vaccination or systemic infection, and can produce antibodies for decades  
• And, intramuscular injection results in improved immune response & less side effects! |

**SLIDE 5** Intramuscular injection is preferred for aluminum-adsorbed vaccines (e.g., diphtheria, tetanus, pertussis, inactivated poliovirus, hepatitis A and B vaccines), because superficial administration leads to increased incidence of local reactions. Both the injection technique and the needle length are crucial for ensuring proper delivery and are directly related to vaccine safety and immunogenicity. However, recent studies have investigated vaccines injected into the skin as intramuscular injection bypasses the skin's immune system into muscle tissue that has no important resident population of antigen-presenting cells. Intradermal injection delivers antigen directly into the skin, which contain large numbers of antigen-presenting cells and has the potential for greater immunogenicity than intramuscular injection.
Active Immunity
• Principle of vaccination: introduce harmless antigen(s) with epitopes also found on the pathogen
• The immune system develops its own immunity against the pathogen
• Vaccines usually attempt to replicate natural immunity to a disease
  • Food for thought: Any recovered AIDS patients?
  • Must use new vaccination strategies for microbes that know how to avoid the immune system or mutate quickly — maybe YOU will discover how

Vaccinations result in active immunity. Active immunity is preferred to passive immunity, as the immune system will develop specificity for the antigen and memory.

Types of Vaccines
• Live attenuated organisms
• Killed/inactivated organisms
• Surface antigen subcellular fragments
• Toxoid
• Recombinant DNA vaccines (experimental)

These 5 vaccine types will be discussed. Also note the comparison of 3 of these types, especially which type require boosters & what type of immunity is produced.

Types of Human Vaccines
• Vaccines can either use the whole organism or pieces of it to stimulate immunity
• Vaccines must be safe, generate specific long-lasting immunity, stable for storage, and inexpensive

Table that illustrates the type (or class) of vaccine for common human vaccines.
Live Attenuated Vaccines

- Organism is infectious, but is treated so that it does not cause disease
- Attenuated: the organism is repeatedly passaged to diminish virulence
  - Poliovirus (Sabin OPV)
  - Measles, mumps, rubella (MMR)
  - Chickenpox (varicella)
  - BCG (tuberculosis)
  - Vaccinia
  - Anthrax
- Highly effective, vaccine most like the "real" agent
- May revert by mutation and regain virulence (Polio OPV)

SLIDE 9 Live, attenuated vaccines are made by weakening a live microorganism by aging it or altering its growth conditions (attenuation). Attenuated vaccines are often the most successful, probably because they multiply in the body thereby causing a large immune response. Immunity is often lifelong and booster shots are not needed. However, these live vaccines also carry the greatest risk because they can mutate back to the virulent form at any time. Reversion to a virulent state would result in induction of the disease rather than in protection against it. For this reason, attenuated vaccines are not recommended for immunocompromised patients. An alternative approach is to use an organism which is similar to the virulent organism but that does not cause serious disease. In 1796, Edward Jenner founded modern immunization practices, when he performed this technique by using the relatively mild cowpox virus to protect against the similar, but often lethal, smallpox virus.

Killed/Inactivated Vaccines

- Organism is not infectious, either killed or treated to remain inactive
- Need multiple doses to maintain immunity
- Poliovirus (Salk IPV)
- Hepatitis A
- Rabies
- Typhoid
- Perusssis

SLIDE 10 Inactivated or killed vaccines remove the risk of mutation back to a virulent form. One popular method to create these vaccines uses formalin to inactivate the microbe. Killed or inactivated vaccines stimulate a weaker immune response than live vaccines. Booster shots are often needed to maintain immunity.
SLIDE 11 Subunit vaccines are created by biotechnology and genetic engineering techniques, which use only the parts of an organism yet also stimulate a strong immune response. To create a subunit vaccine, researchers isolate the gene or genes which code for immunogenic subunits from the genome of the infectious agent. This genetic material is placed into bacteria or yeast host cells which then produce large quantities of subunit molecules. These subunit molecules can be isolated, purified, and used as a vaccine. Subunit vaccines are safe for immunocompromised patients because they cannot cause the disease. The antigenic portion of the infectious organism can also directly be used to create a vaccine. These acellular vaccines may only contain the capsule, the flagella, or part of the protein cell wall. Acellular vaccines exhibit some similarities to killed vaccines: neither killed nor acellular vaccines generally induce the strongest immune responses and may therefore require a "booster" to have continued effectiveness. Acellular vaccines are safe for use in immunocompromised patients.

SLIDE 12 Some vaccines are made from toxins. The toxin is often treated first with aluminum or adsorbed onto aluminum salts to decrease its harmful effects; after such treatment the toxin is called a "toxoid". Toxoid vaccines often induce low level immune responses and are therefore sometimes administered with an "adjuvant" to improve the immune response. Toxoid vaccines often require a booster every ten years.
Recombinant DNA/Experimental

- Immunization uses the genes for antigens, rather than the antigens themselves, as the source of immunogen
- DNA sequences encode one or more protein antigens or epitopes of the complete organism
- DNA vaccines can be injected into a muscle just like conventional vaccines.

**SLIDE 13** DNA vaccines are an experimental technique that may actually trick the body into believing it is infected with the real thing, as our own cells will make the foreign proteins. This will result in both cell mediated & antibody mediated immunity, unlike most vaccines which only generate antibodies.

Recombinant DNA/Experimental

- These vaccines are very promising as they:
  - Elicit cell-mediated & antibody-mediated immune responses (most vaccines just stimulate AMI)
  - Decreased side effects
  - Cannot revert to wild type organism
- Limitations:
  - Person may already have antibodies to the vector (usually an innocuous virus) used to introduce the DNA, which will neutralize the immunization
  - May not elicit a strong enough response, might need "boosters"
  - DNA vaccines for HIV-1 already in clinical trials (over a dozen!)
  - DNA vaccines for tuberculosis, SARS, & smallpox have shown promise in mouse studies

**SLIDE 14** These vaccines will result in both cell mediated & antibody mediated immunity, unlike most vaccines which only generate antibodies. However, some limitations exist in that the person may already have antibodies to the virus used to inoculate the DNA. These will neutralize the vector virus & prevent the DNA for the new vaccine from entering the cell.

Recommended immunization schedule for children. Note that most of the vaccines should be given before 2 years of age, and then booster doses should be given in later years.
**Slide 16**

Recommended immunization schedule for adults. Booster doses for some vaccines are recommended every 10 years. The influenza vaccine is recommended every year for adults 19-49 at risk for the disease (health care workers, volunteers in nursing homes or hospitals, etc) and for everyone aged 50 or older.

**Slide 17**

Although common childhood diseases (such as measles, diptheria, & mumps) no longer occur in the United States because of vaccination, this is not the case in other countries. Consequently, it is important to maintain immunization levels at 80% or higher so that herd immunity will limit the number of susceptible people. This is especially important as international travel has increased & these diseases can easily be re-introduced into the US from travelers. In the United States, state laws require proof of vaccination before children enter public school. This safety net ensures that no child is missed. However, the increase in home-schooled children & those attending private schools bypass this safety net and may not be immunized. No system is perfect! The use of biological agents as a weapon by terrorist organizations or governments is a considerable public health threat. For example, anthrax has been weaponized by several governments (including the US) and stockpiles may have been compromised when the Soviet Union collapsed. The same concern applies to smallpox. Although we have a vaccine for both diseases, these vaccines can cause serious side effects. Mass vaccination for smallpox ended in the 1970s after the disease was eradicated globally. No natural cases have occurred since then, and the intentional release of smallpox would constitute a humanitarian disaster. The problem is that now everyone is susceptible to the disease, unless they have been vaccinated in the last 5 years. Now, no herd immunity exists for smallpox & recent vaccination of first responders & military personnel showed that adults that get the vaccine have serious side effects.
Top Killers

- 6 infectious diseases cause 90% of mortality globally related to illness from infectious causes
  1. Acute respiratory infections (may lead to pneumonia)
  2. HIV/AIDS
  3. Diarrheal disease
  4. Tuberculosis
  5. Malaria
  6. Measles

- For only one of these diseases – measles – is there a vaccine that can prevent infection

Public health programs have helped to eliminate many infectious diseases in the developed world. However, infectious diseases are still major killers in other parts of the world where access to health care or medicines are unavailable to treat the illness. Most respiratory infections or diarrheal illnesses are easily treated in the US where modern medical interventions to prevent dehydration or respirators are available. Unfortunately, only measles has a very effective vaccine to prevent infection of these top 6 killers. Malaria, HIV/AIDS, and some viruses that cause diarrheal illness (such as rotavirus) do have vaccines currently in development. The BCG vaccine for tuberculosis is available but is not used in the United States where prevalence of the disease is low. In addition, the BCG vaccine appears to only reduce the severity of the disease in infants and young children, but does not protect them through the adult years & they often later develop disease. Nearly 30% of the global population is infected with TB, despite widespread BCG vaccination since the 1930s. Clearly, further research is warranted to develop an effective tuberculosis vaccine. The WHO has estimated that over 1.4 million of deaths among children 5 or younger were from diseases that could have been prevented by routine vaccination (2002). This is especially tragic in light of the millions of deaths that are caused because there is no vaccine, whereas these deaths are senseless & easily prevented.
SLIDE 19 Based on the success of the polio vaccination campaign in the United States during the 1950s-1960s, elimination of yellow fever in the United States, as well as a successful vaccine for measles led to predictions that infectious diseases (especially viruses) were dead or easily controlled. Scientists believed that we had won the war against infectious diseases forever. Obviously, the optimism of the 1970s soon was challenged with a mysterious disease that alarmed doctors and public health officials in 1981- AIDS. The emergence and establishment of HIV throughout the world’s population over the last 25 years is the most significant threat to global health, and thus far, efforts to create a vaccine have failed. In fact, the emergence or re-emergence of over 30 infectious diseases over the last few years will continue to challenge public health professionals for the rest of the century, as vaccines & prevention strategies are developed to contain these diseases. The emergence of these diseases will require new technologies, further insight into the body’s immune response to these organisms, and the development of novel vaccines to ensure protection for the global community through immunizations. One current example of the practice of immunology in public health is the development and trials for an avian influenza vaccine.

SLIDE 20 Despite the best efforts of the humoral immune response, some microbes have figured out ways to beat the immune system and hide from antibodies. The following slides will detail some of these crafty bugs and the strategies they use to beat the odds & make us sick!
SLIDE 21 Several important limitations of vaccines are shown here.

- Most vaccines protect against disease, not infection (except viruses that enter through blood, mucosal or respiratory tract, where Abs can neutralize).
- Most vaccines protect against infrequently encountered infections.
- Most vaccines generate antibody response, not cell-mediated.
- Best response is to live organisms, which generate both humoral & cell-mediated response.

SLIDE 22 The humoral immune response is dependent on antibody production. However, antibodies are made to antigens on the surface of organisms. Many microbes can rapidly mutate and change these antigens, which will not be "remembered" when the same organism infects you again. This is because these organisms have evolved in response to selective pressures to eliminate them to rapidly mutate their genome & change the appearance of their antigens.

- Viruses hide inside cells.
- Antibodies only work extracellularly.
- Human immunodeficiency virus (HIV) infects & kills immune cells – direct strategy to decrease the immune response.
- Retrovirus integrates into host genome & latency.
- Rapidly mutates RNA to create new virus variants inside the body – antibodies one step behind.
- Antibodies are made to epitopes, not genome, and rapidly changing microbes have the advantage.

SLIDE 23 If more than one serotype of dengue virus infects you (there are 4 strains), the likelihood of dengue hemorrhagic fever and serious complications, including death, increases due to the virus using antibodies to enhance uptake. Each serotype has its own antigenic character which induces a different antibody to each type. In this case, antibody dependent enhancement (ADE) protects against one serotype (and future infections of that same type), but can enhance the infection (i.e. make the disease more severe) of a different serotype. In 2005, dengue is the most important mosquito-borne viral disease affecting humans, with a global distribution comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission. Staph aureus also has surface factors that prevent phagocytic engulfment, such as a capsule and Protein A. Protein A binds IgG by its constant region in a manner opposite of its normal orientation. This disguises the microbe in the IgG molecules and makes it extremely difficult for phagocytes to eat and destroy the bacteria since they can no longer use the antibody as an opsonin.
Slide 24

**Immune Evasion Strategies: Malaria**

- Malarial parasite prevents both antibody & cell-mediated immunity
- Multiple life stages, in different cell types
- Changes surface antigens & depletes T cells
- Always one step ahead of immune system

SLIDE 24 The malarial parasite (Plasmodium species) is very adept at manipulating the system. First, it lives in several cell types, including mature red blood cells that lack a nucleus. This is very important as mature RBCs are not protected by T cells. Consequently, the parasite can replicate in these cells & then burst to release thousands of parasites to infect other cells without fear of being killed by the cell mediated branch of the immune system. In addition, the parasite has several life stages, with different antigens, lives in multiple cell types, and can change its surface to evade the immune response.

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Slide 25

**Antigenic Variation**

- Microbes change their surface proteins to evade the immune response
- Influenza virus alters the hemagglutinin and neuraminidase proteins on its surface (antigenic drift & shift)
  - New human flu vaccine every year
  - Antigenic shift is a large change in the H or N proteins, no one will have immunity
  - Major change = pandemic!

SLIDE 25 Antigenic variation is a very popular choice of immune evasion strategy for several microbes. This type of variation keeps the organism one step ahead of the antibody response, so that each time the antigen changes, a whole new round of clonal selection & expansion will have to occur. Influenza virus alters the proteins on its surface every year, which makes it necessary to create a new vaccine based on the current circulating strains. The H5N1 avian influenza virus is such a threat to human health as no one has any antibodies to protect against this strain. From year to year, we build up a repertoire of B cell clones & antibodies to strains that are similar to each other. This is termed antigenic drift, when the virus only mutates a little and the surface antigens are the same type, & most people will still have some protection from previous years. However, if the virus mutates a lot, this is termed antigenic drift & no one will have protection from previous exposures, as the virus will be “new". This is the concern with an avian flu pandemic, as no one has been exposed to the H5N1 strain. For now, the avian flu virus has not mutated enough to easily cause disease and be spread by humans. Most cases are from people that had close contact with birds, but if the virus mutates so that it can be spread by the respiratory route in people, then a pandemic is likely.
SLIDE 26 An avian flu pandemic is a significant public health threat. Currently, no vaccine exists for avian flu. It is possible that people that receive the human flu vaccine every year may have slight protection from the disease, as they have antibodies to influenza. Yet, it is believed that this will not be sufficient protection for the highly virulent H5N1 strain of avian flu. Stockpiles of TamiFlu (an anti-viral drug) and experimental avian flu vaccines will not be sufficient for the population, and will be rationed according to need. As the virus infects more people, it is mutating and changing from the bird strain. The concern is that if the avian version of the virus also infects a person or pig with the human strains of the virus, it may pick up the genes necessary for respiratory transmission. Every year, the human influenza virus (& vaccine) changes because of the ability of the flu virus to mix in several species, including pigs, birds & mammals. This mixing of the virus in these animals generate new strains of the virus, changing the antigenic makeup of the hemagglutinin & neuramindase proteins (antigens) on the surface of the virus. Consequently, a new vaccine is produced every year to create new antibodies to these changing antigens. It is believed that an avian flu pandemic is inevitable. Make sure to watch the avian flu video segments this week to see how cultural practices will contribute to an avian flu epidemic.

SLIDE 27 Trypanosoma brucei is also a master at antigenic variation. Unlike most bacteria and viruses, parasites can live inside a person for years and sometimes a lifetime. They need us to survive, and have developed very advanced strategies for outsmarting the immune system so that they can live in peace. T. brucei is especially good at antigenic variation, so that it changes its surface antigens every week to evade antibodies.
**Slide 28**

**Constant Battle**
- How do you defend when the microbes are rapidly changing?
- Immune response does not change – strict regulation to prevent attack of self antigens
- New vaccines (HIV, malaria) must be smarter than the microbes
- So far, none have been successful!

**Slide 29**

**In Summary**
- Principles of vaccination, herd immunity
- Types & examples of vaccines
- Emergence of infectious diseases
- Public health challenges to vaccinate every child
- Vaccine limitations
- Examples of immune evasion strategies

**Slide 30**

**Self-Test Questions**
- Describe how a vaccine can stimulate immunity.
- What are the 5 types of vaccines? Provide an example of each type.
- What type of vaccine stimulates both antibody & cell mediated immunity?
- What type(s) of vaccines require booster shots? Why?
- What is herd immunity?
- What is antigenic variation? What organisms practice this technique to evade the immune response?