Chapter 8

Prevention of Infectious Disease

In Chapter 7, we examined the process of designing new technologies and the interdisciplinary translational research efforts needed to advance technologies from the laboratory to clinical practice. In the rest of Unit 3, we will explore in detail the development of several types of new technologies which draw on advances in the different sub-disciplines of bioengineering. We begin by focusing on the development of vaccines to prevent infectious disease. We will see that scientific knowledge, such as an understanding of both the organisms that cause disease and the protective mechanisms of the immune system, is critical to enable the engineering of preventive vaccines. We will examine the development of vaccines from idea to product.

We have seen that infectious diseases are responsible for a large fraction of deaths, particularly in the developing world. In high-mortality developing countries, infectious disease is responsible for nearly half of all deaths, and the childhood cluster diseases (pertussis, poliomyelitis, diphtheria, measles, and tetanus) kill just over half a million children under the age of 5 each year.[2] In the developed world, the use of vaccines has dramatically reduced the incidence of infectious disease. As we explore how vaccines work, we will trace the tremendous improvements in world health that have resulted from mass childhood immunization and examine the global obstacles that remain. There are many diseases for which no vaccine is available. We conclude this chapter by considering the scientific and engineering challenges associated with developing new vaccines to prevent HIV infection.

The Immune System

Vaccines manipulate the immune system of the recipient; thus to understand how vaccines work, we must first understand how the immune system prevents and fights infec-

“The gasping breath and distinctive sounds of whooping cough; the iron lungs and braces designed for children paralyzed by polio; and the devastating birth defects caused by rubella: To most Americans, these infectious scourges simultaneously inspire dread and represent obscure maladies of years past. Yet a little more than a century ago, the US infant mortality rate was a staggering 20%, and the childhood mortality rate before age 5 was another disconcerting 20%...Fortunately, many of these devastating diseases have been contained, especially in industrialized nations, because of the development and widespread distribution of safe, effective and affordable vaccines.”

In Chapter 1, we examined the use of high dose chemotherapy for breast cancer. A side effect of that treatment was that it destroyed cells in the patient’s bone marrow, leaving patients vulnerable to infection. Stem cells in the bone marrow are responsible for generating the cellular components of blood: the oxygen carrying red blood cells, the platelets which aid in blood clotting and the infection-fighting white blood cells. There are five main types of white blood cells: eosinophils, neutrophils, basophils, lymphocytes and monocytes (Figure 8.2). Eosinophils are important in fighting infections due to parasites, such as malaria. Neutrophils are important in fighting infections caused by bacteria, such as tuberculosis. Monocytes leave the blood stream and mature into macrophages, which are also important in fighting bacterial infections. As we will see, lymphocytes are important in the fight against both bacterial and viral infections. The function of basophils is poorly understood, but they are believed to be important in allergic reactions. To understand how white blood cells work as part of the immune system to fight infection, we next examine how infectious agents, such as bacteria and viruses, cause disease.[3]

**How Infectious Agents Cause Disease:**
Bacteria and viruses cause disease in very different ways.
Bacteria consist of cells with a cell membrane and, unlike human cells, usually also have a cell wall (Figure 8.3). Bacteria can survive outside of a host and are capable of reproduction without a host. Bacteria can be killed or inhibited by antibiotics, which frequently destroy the bacterial cell wall. Viruses consist of a nucleic acid core surrounded by a protein envelope (Figure 8.4). Unlike bacteria, viruses must use the intracellular machinery of their host to reproduce, and they cannot be killed with antibiotics. There are more than 50 different viruses that can infect humans.[4]

Whether virus or bacteria, there are three basic problems each pathogen must solve: (1) How to reproduce inside a human host, (2) How to spread from one person to another, and (3) How to evade the immune system. Because of their fundamental structural and functional differences, bacteria and viruses solve these problems and cause disease in very different ways. Bacteria invade a host, and then begin to reproduce. As they grow and reproduce, they produce toxins which disturb the function of normal cells. Viruses actually invade the cells of their host (Figure 8.5). They typically accomplish this invasion by binding to receptors on the membrane of the host cell, which then transport the virus into the cell in a process known as endocytosis. Once inside, the virus takes over the cell, using viral nucleic acid and host cell resources to make new viral nucleic acid and proteins. As the virus directs the synthesis of new viral particles, more virus is released from host cell. The new virus is disseminated when the virus either causes the host cell to lyse (break apart) or the newly formed viral particles bud from host cell surface.[3]

**Bacterial Disease:** Let’s examine a once common bacterial
At the end of a pertussis coughing episode, a long gasp is accompanied by a high pitched sound (whoop). You can listen to a typical “whooping” cough associated with pertussis at:
http://www.whoopingcough.net/sound%20of%20whooping%20cough%20with%20some%20whoop.htm

Before the availability of the pertussis vaccine in the 1940s, more than 200,000 cases of whooping cough were reported each year in the US. The disease is still an important cause of mortality in developing countries; in 2001, pertussis was estimated to cause more than 285,000 deaths in children in developing countries.[5]


Viral Disease: In contrast, viral pathogens have developed different ways to reproduce in a host, spread from one person to another and evade the immune system. How does a common virus like influenza solve these problems? In order to reproduce, the influenza virus must get inside the human cell to use the cell’s biosynthetic machinery. Influenza virus accomplishes this by binding to receptors on the host cell surface and then inducing receptor mediated endocytosis. Once it has been endocytosed, the influenza virus is trapped in a vesicle made of the cell membrane called an endosome. The virus acts to slowly reduce the pH in the endosome, creating a hole in the membrane through which the virus releases its single stranded RNA and polymerase proteins. These RNA segments and polymerase proteins enter the nucleus of the infected cell and direct the cell to begin making many copies of the viral RNA and viral coat proteins. These new viral particles then exit the nucleus and bud from the cell. During this reproduction, the viral polymerase proteins don’t proofread reproduction, and as a result nearly every virus produced in an influenza-infected cell is a mutant, differing slightly from the original infecting virus. [4]
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How does the influenza virus spread from one person to another? Generally, this happens when an infected person sneezes or coughs (Figure 8.7), and micro-droplets containing viral particles are inhaled by another person. The influenza virus is particularly adept at penetrating epithelial cells lining the respiratory tract, killing cells that it infects. The resulting inflammation triggers a cough reflex to clear airways of foreign invaders. During influenza infection, the immune system produces large quantities of a substance called interferon. Interferon leads to the common symptoms of the flu: fever, muscle aches, headaches and fatigue.[4]

Let's look at a more deadly pathogen - the HIV virus. HIV consists of a central core of RNA and an enzyme called reverse transcriptase surrounded by a protein core (Figure 8.8). The entire virus is surrounded with a lipid membrane; this membrane is studded with special proteins called gp120 envelope proteins that enable it to bind to the surface of host cells. In particular, this envelope protein is recognized by receptors on the surface of a special type of lymphocyte. [3]

Figure 8.9 illustrates what happens when the HIV virus binds to the surface of a host lymphocyte. The membrane of the viral particle fuses with the host cell membrane, allowing the contents of the viral particle to enter the cytoplasm of the lymphocyte. The viral enzyme reverse transcriptase uses the host machinery to turn the viral RNA into viral DNA. The viral DNA then directs the lymphocyte to produce new copies of viral protein and RNA which are assembled into protein coated viral particles within the lymphocyte. These mature particles can then bud out from the cell to release new copies of the virus into the host. Thus, HIV infection destroys an important component of the immune system; without treatment, patients develop AIDS and severe immunodeficiency. Patients with severely compromised immune function become susceptible to a variety of opportunistic infections which can result in death.[3]

How the Immune System Fights Pathogens: How are we protected from bacterial and viral attack? Evolution has provided two simple protective strategies: (1) keep pathogens out and (2) kill them if they get in. To accomplish these goals, we are protected by three layers of immunity (Figure 8.10). First, physical barriers act to keep pathogens out. The most important physical barriers are the skin and mucous membranes. These barriers must defend an enormous area – humans have over 2 square meters of skin and 400 square meters of mucous membranes.[6] Second, all animals possess an innate immune system to fight patho-

Figure 8.7: Influenza is spread from person to person through coughs or sneezing.

Figure 8.8: The HIV virus.

Figure 8.9: Life cycle of the HIV virus.
gens that make it past these physical barriers. The innate immune system recognizes molecular patterns typically associated with pathogens and responds with a general inflammatory response to fight those pathogens which penetrate physical barriers. Thirdly, vertebrates possess an adaptive immune system, capable of recognizing and adapting itself to defend against any invader. The adaptive immune system becomes important when the innate immune system cannot defend against attack. The adaptive system also provides the immune system with "memory".[3]

Figure 8.11 shows a microscopic view of a protective physical barrier – in this case, the mucous membrane lining of the uterine cervix. This lining is about 250 microns thick (about the diameter of a human hair) and consists of multiple layers of specialized epithelial cells. The epithelial cells at the bottom of the layer are rapidly dividing and are responsible for regenerating the epithelial tissue as it dies. As we move toward the surface of the epithelium, the cells become more mature. Cells at the very top layer are dead, but the tight junctions between these cells provide an important barrier which is difficult for many pathogens to cross. In addition, substances present on the surface of many mucus membranes and the skin provide a chemical barrier, which functions to trap pathogens and may contain enzymes or other molecules with anti-bacterial activity.

What happens when a pathogen is able to cross this barrier? You have no doubt experienced this if you have ever gotten a splinter (Figure 8.12). In this case, a sharp piece of wood crosses the skin, enabling bacteria to penetrate beneath the epithelial lining. What are the symptoms you experience? Frequently the area can become red, swollen, and warm. Sometimes the area will ooze pus. These symptoms are all signs that the second line of defense—the innate immune system—is kicking into gear. In most cases, the innate immune system can respond to the pathogen. A specialized kind of cell called a macrophage continually patrols beneath the epithelium, to detect foreign invaders and signal the immune system to respond. Macrophages are derived from monocytes, one of the 5 types of white blood cells. You can think of the macrophages as guards that patrol just beneath the physical barriers (skin and mucous membranes). When macrophages encounter bacteria on a splinter they ingest the bacteria in a process known as phagocytosis (Figure 8.13). When macrophages are activated in this manner, they produce chemicals which increase local blood flow. It is this increase in blood flow that makes the area around the splinter appear red and warm. These chemicals
also cause the blood vessels in the area to become leaky. Fluid leaking out of the blood vessels produces the swelling around the area with the splinter. The chemicals released by the macrophages also recruit other white blood cells, such as neutrophils, to the site of the infection. Neutrophils aid in phagocytosing bacteria, and these cells make up the pus which can sometimes be present in the area.[6]

The innate immune system is primarily effective against pathogens outside of cells. How do macrophages recognize extracellular invaders as foreign? Macrophages recognize foreign invaders in two ways. There are particular molecular patterns found on the surface of many pathogenic microorganisms. These signatures are recognized by receptors on the surface of the macrophage. In order to evade this route of detection by the immune system, many bacteria have evolved to hide their surface markers behind a polysaccharide capsule.[3] In some cases, proteins within the blood of the host can bind to pathogens and mark them for destruction by macrophages as a way to guide the innate immune system. In any case, if macrophages identify an invader, they become activated. Once activated they send signals to recruit other immune system cells (neutrophils), they become vicious killers, and they activate the third line of defense, the adaptive immune system. [6]

The adaptive immune system has two main components. The first component, called humoral immunity, relies on large proteins called antibodies (Figure 8.14) to recognize and fight pathogens outside of cells. The second component, called cell-mediated immunity, relies on several types of white blood cells to kill pathogens inside of cells. The innate immune system recognizes general molecular signatures of pathogens and provides a generalized response. In contrast, the adaptive immune system recognizes specific molecular signatures called antigens, associated with individual pathogens. The two components of the adaptive immune system accomplish the recognition using different strategies. The humoral component of the adaptive immune system relies on the chemical specificity of antibodies to recognize different pathogens. Recognition by the cell-mediated component of the adaptive immune system is facilitated by specific receptors on the surface of lymphocytes. [3]

Let’s first examine how antibodies help to recognize and kill pathogens. Antibodies are Y-shaped proteins, about 12 nm in length, which are made by the immune system. The bottom of the Y is known as the Fc region, while the top of the Y has two antigen binding sites (Fab region) which can bind

This response is known as inflammation, and results in the redness, swelling, heat and pus that are sometimes present at the site of an infection.

Figure 8.13: A macrophage ‘eats’ a bacteria.

When activated, macrophages recruit circulating neutrophils to the site of inflammation. The video shows neutrophils rolling along the surface of a venule; macrophages secrete chemicals which cause neutrophils to exit vessels and enter tissue. Follow the link below for video microscopy of neutrophils rolling along a blood vessel.

http://www.cbrinstitute.org/labs/springer/lab_goodies/lab_goodies.html
either to the surface of a free bacteria or virus or to the surface of a virus-infected cell.[4, 7] The free Fc region hanging off the pathogen then binds to macrophages and neutrophils and induces them to phagocytose the tagged pathogen. The Fc region can also bind to a special kind of lymphocyte known as a natural killer cell to induce destruction of the invader.[4] Essentially, you can think of an antibody as a bridge between a pathogen and the tool to kill it. The Fab portion of the antibody recognizes the antigen while the Fc region interacts with other components of the immune system to initiate destruction of the pathogen.

Antibodies are made by a type of lymphocyte called B cells. These B cells have special receptors on their surface that are designed to recognize foreign pathogens. We all have 100 million different types of B cells, each with different surface receptors. These B cell receptors are so diverse they can recognize every organic molecule; thus, they provide the ability to recognize specific pathogens.[4] When a B cell binds an antigen it begins to rapidly divide and proliferate - in one week, a clone of 20,000 identical B cells can be created.[6] This process is known as clonal expansion (Figure 8.15). Each of these B cells secretes antibodies which will recognize the specific pathogen targeted by that B cell.

Antibodies are helpful to recognize and target pathogens outside of cells. However, many viruses hide inside our cells. How do we kill viruses once they are inside the cell, where antibodies cannot reach them? This process is carried out by a special class of white blood cells called T lymphocytes. T cells recognize protein antigens. Again, we require a way to let T cells know which cells have been invaded by viruses. All of the cells in your body have special molecules on their surface known as major histocompatibility (MHC) molecules. These molecules help your immune system recognize the cells of your body as ‘self’ so that they do not come under attack by the immune system.[7] When a virus invades a cell, fragments of viral proteins are loaded onto MHC proteins (Figure 8.16). T cells inspect the MHC proteins on the surface of your cells and use this as a signal to identify infected cells (Figure 8.16) and target them for destruction. Like B cells, when T cells bind antigen, they undergo clonal selection.[4]

The process of clonal expansion enables the adaptive immune system to have memory (Figure 8.17). The first time the adaptive immune system is activated by an antigen, your body builds up a clone of B cells and T cells. This process takes about a week.[6] After the infection is over, most of these cells die off; however, some cells, known as mem-
ory cells, remain. If the immune system is activated a second time by the same antigen, these memory cells are much easier to activate (Figure 8.18). The response of the immune system is much faster, generally so much more rapid that you don’t experience any symptoms associated with the infection. [6] This memory explains why you are ‘immune’ to most diseases after a first exposure (think chicken pox) and is also what allows us to develop vaccines to establish this immunity in a safer way.

Why then do we get the flu more than one time? Influenza virus particles are usually 80—120 nm in diameter, and consist of an outer lipid envelope, an intermediate protein capsid, and a central core of RNA. There are three major types of influenza virus: A, B, C. Most serious cases of the flu in humans are caused by type A influenza. Figure 8.20 shows a schematic drawing of an influenza virus; there are two kinds of proteins are found in the lipid envelope of the influenza virus. Hemaglutinin mediates attachment of viral particles to the host cell membrane; neuraminidase mediates release of newly formed viral particles from host cell. Type A virus contains 8 single stranded pieces of RNA.[3]

The influenza virus can evade immune extinction in two ways: antigenic drift and antigenic shift. As influenza reproduces, reproduction errors occur that change the structure of the virus. The changes are so slight that the virus is still capable of infection and reproduction but significant enough that the immune system does not possess memory for the changed virus. Mutations in the virus have resulted in a number of different strains of influenza virus, and these are characterized by differences in the two major coat proteins, such as influenza A (H2N1).[3] This antigenic drift is why the structure of the flu vaccine changes annually.

Antigenic shift refers to much larger changes in the structure
Antiviral drugs are effective against the influenza virus if taken within 2 days of the onset of symptoms. Many of these drugs work by hindering the change in pH necessary for influenza virus to escape the endosome following endocytosis. Other antiviral drugs block the effect of neuraminidase, to inhibit release of new viral particles. H5N1 is resistant to some antiviral drugs, but responds to others.

Mortality due to influenza fluctuates seasonally. The CDC tracks mortality, to monitor for epidemics of influenza, where the mortality rate rises above the seasonally anticipated baseline level. In 2004, an influenza epidemic occurred in the US.

![Image of influenza virus]

**Figure 8.20**: The influenza virus.

![Graph of Pneumonia and Influenza Mortality rates for years 2001-2005](http://www.cdc.gov/flu/weekly/weeklyarchives2004-2005/images/bigpiccurvesumary04-05.gif)

**Figure 8.19**: Pneumonia and influenza mortality rates for years 2001-2005.

of the virus. Sometimes animals can be co-infected by different strains of the influenza virus. During viral replication, viral gene segments from different influenza strains are present inside the same cell; occasionally, these gene segments can randomly reassort to create a completely new strain of the virus. Reassortment can lead to new strains of influenza that are capable of infecting humans. If a new strain can be easily transmitted from person to person, an influenza pandemic (global outbreak) can occur because people do not have any immunity to this new strain.[3]

As a result of genetic shift, there have been 3 pandemics of flu in the last century (Figure 8.21). The largest occurred in 1918, when the Spanish flu (H1N1) affected between 20-40% of world’s population, killing more than 50 million people worldwide and 675,000 in the US.[9, 10] The virus had a particularly high mortality rate in young adults. The impact of this pandemic on the US was so significant that life expectancy dropped by 10 years.[10] Why did the Spanish flu kill so many? To gain insight into why this virus was so lethal, scientists recently reconstructed the H1N1 influenza virus and used it to infect macaque monkeys; the experiment was carried out in a biosecure facility.[11] They found that the virus triggered a severe immune reaction in the animals; the immune reaction was so severe that it provoked the body to begin killing its own cells and could rapidly destroy large amounts of lung tissue, leading to death.

Today, there is particular concern regarding the strain of the flu known as avian flu, or influenza A(H5N1). This strain of flu is ordinarily carried by wild birds and does not grow well in human cells. The virus is spread from bird to bird through fecal contact, and is very contagious among birds. While it usually does not cause illness in wild birds, it can quickly devastate populations of domesticated birds—with a mortality rate approaching 100%.[9] H5N1 does not normally
infect humans, but in rare cases it can be transmitted from birds to people. Over the last few years, several hundred cases of H5N1 have been reported in humans, mostly in Asia. Fatality rates have been reported to be approximately 50%.[12] The majority of these cases were acquired via bird to human transmission; however, a few cases of human to human transmission have been reported.[13] Today, there is considerable concern that the H5N1 will mutate into a highly infectious strain that can spread rapidly from person to person. This could lead to a pandemic with potentially very high mortality. Later we will examine the challenges associated with preventing such a pandemic.

Summary:
The genetic simplicity of many infectious pathogens allows them to undergo rapid evolution; our immune system must constantly cope with pathogens that develop selective advantages to avoid detection and destruction by the immune system. The complexity of the immune system is remarkable, and we have seen only an overview of how this system functions to protect us from disease (Figure 8.22). Physical barriers prevent many pathogens from entering tissue. The innate immune system provides a rapid response system to detect and attack many extracellular pathogens that make it past these physical barriers. The adaptive immune system provides the diversity to recognize over 100 million antigens and to remember which have been encountered previously. Antibody-mediated immunity can fight pathogens outside of cells, and cell-mediated immunity can fight pathogens within cells. In the next section, we examine how we can exploit the properties of the adaptive immune system to provide immunity to dangerous pathogens in a process called vaccination.

How Vaccines Work
Vaccines are the most cost-effective medical intervention known to prevent death or disease. Vaccination is the practice of manipulating the adaptive immune system to artificially induce immunity. The goal of vaccination is to stimulate the adaptive immune system to make memory cells that will protect the vaccinated person against future exposure to pathogen, without causing the symptoms of disease.

How can we stimulate the adaptive immune system to make memory cells? It is easiest to stimulate humoral immunity because this component of the immune system responds to extracellular pathogens. In order to create memory B cells, we simply need to expose B cell receptors to the pathogen or some part of the pathogen. It is more difficult to stimulate cell mediated immunity because this component of the im-
Prevention of Infectious Disease

There are several types of vaccines which can stimulate the adaptive immune system to provide memory and protect against future exposure to a pathogen. Here, we will examine three types of vaccines including: (1) inactivated organism vaccines, (2) live attenuated vaccines, and (3) pathogen subunit vaccines. Table 8.1 compares the advantages and disadvantages of each of these strategies.

**Table 8.1[5]:** Advantages and disadvantages of three types of vaccines.

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-infectious vaccines:</strong></td>
<td>• Stimulates humoral immunity</td>
<td>• Does not stimulate cell mediated immunity</td>
</tr>
<tr>
<td>Vaccinate with killed pathogen</td>
<td>• Minimal danger of infection</td>
<td>• Usually need booster vaccines</td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Live attenuated bacterial</strong></td>
<td>• Stimulates both humoral and cell mediated immunity</td>
<td>• Poses some risk of disease, particularly in immunocompromised host</td>
</tr>
<tr>
<td>or viral vaccines:</td>
<td>• Usually provides life-long immunity</td>
<td></td>
</tr>
<tr>
<td>Vaccinate with weakened pathogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subunit vaccines:</strong></td>
<td>• Stimulates humoral immunity</td>
<td>• Does not stimulate cell mediated immunity</td>
</tr>
<tr>
<td>Vaccinate with pathogen sub-unit</td>
<td>• Minimal danger of infection</td>
<td>• Usually need booster vaccines</td>
</tr>
</tbody>
</table>

The immune system responds to intracellular pathogens. Memory killer T cells are only created when antigen presenting cells are infected with a pathogen. Our goal in making a vaccine is to provide this exposure in a safe way.

For example, pathogens can be treated with chemicals (like formaldehyde) to kill them. When a patient is exposed to the dead pathogen, they mount an immune response without becoming infected. The Salk (inactivated) Polio vaccine, the hepatitis A vaccine, and the rabies vaccine are examples of killed pathogen vaccines.[5] The immune system encounters the pathogen outside of cells, so that this approach stimulates humoral immunity. Because the pathogen has been killed it does not infect cells, so this approach does not stimulate cell-mediated immunity. As a result, booster vaccines are usually required to maintain lifelong immunity to these diseases. When using vaccines based on an inactivated organism alone, the vaccine may not stimulate immune system sufficiently. In order to increase the response of the immune system, the vaccine is sometimes formulated with an adjuvant—a substance that increases the response.
of the immune system. Aluminum salts are frequently used as an adjuvant in vaccines.[14]

**Live Attenuated Vaccines:** A stronger immune response can be produced by vaccinating with a live pathogen which has been weakened so that it does not cause disease, but still elicits immunity. In this approach, the pathogen is grown in host cells in the cell culture laboratory in order to produce mutations which weaken the pathogen so it cannot produce disease in healthy people, but can still produce a sufficiently strong immune response that protects against future infection. The Sabin Polio vaccine (oral Polio), measles, mumps, rubella (MMR), and varicella vaccines are examples of vaccines made using this approach.[5] This approach has a number of advantages. Live, attenuated organism vaccines contain the target antigens for humoral antibody, the pathogen molecular patterns for stimulating innate immunity, and, because of the invasiveness of the organism, it can deliver antigens effectively.[14] The immune system treats live, attenuated vaccines in just the same way as it would an infectious pathogen. Thus, these vaccines stimulate both humoral and cell-mediated immunity. As a result, they usually provide life-long immunity. However, such vaccinations can produce disease in an immunocompromised host.[5]

A technical challenge of this approach is that it is not always feasible to produce strains of a pathogen that have been attenuated sufficiently. In addition with pathogens that undergo antigenic drift, there is a finite risk of reversion to a virulent form.[14] Vaccines based on live attenuated pathogens account for approximately 10% of total vaccine sales.[15]

**Subunit Vaccines:** Our immune system generally recognizes and responds to a portion of an infectious

**Edible Vaccines:** Subunit vaccines can also be produced in plants; genes are introduced which induce the plant to make the protein that will stimulate immunity. Usually subunit vaccines are expensive because you must purify the protein grown in culture. With plants, you don’t have to do this. Plants can be grown locally, avoiding problems with vaccine transport. Usually subunit vaccines are injected, because the digestive system will destroy the protein in the stomach before it can be presented to the immune system. However, in plants, the protein is protected by the cell walls of the plant cells. As a result, the protein survives until it reaches the intestine, where immune cells in the intestinal wall are activated.

Studies have shown that tomatoes and potatoes can synthesize antigens from major causes of diarrhea: Norwalk virus, enterotoxigenic E. coli, and Vibrio cholera. Feeding these plants to animals can evoke immune response, and provide partial exposure to the real toxin. Small trials in human volunteers have produced immune reactivity in people.

Challenges: Scientists are grappling with the problem of getting the plants to produce a sufficient amount of antigen. When plants produce large quantities of antigen, they tend to grow poorly. Also, some plants require cooking in order to be palatable. Plants containing edible vaccines cannot be cooked because heating denatures the proteins, thereby preventing an appropriate immune response.
organism known as an antigen. If we can identify the antigen that will produce an immune response, we can purify that antigen and use it as the basis for a vaccine. This type of vaccine is very safe because there is no risk that it can lead to infection, even in an immunocompromised host.[5] Several strategies have been developed to produce subunit vaccines. For example, many bacteria produce disease by secreting toxins that interfere with normal cell function. We can create an immune response to these toxins by vaccinating with purified bacterial toxins that have been chemically treated to make them harmless. This type of vaccine is known as a toxoid vaccine and it produces an immune response without symptoms of disease.[14] The diphtheria and tetanus vaccines are examples of toxoid vaccines.[5] Alternatively, a subunit vaccine can use part of a pathogen to induce an immune response but not disease. Our immune system responds to the polysaccharides found on the surface of certain bacteria. We can grow bacteria in culture and extract these polysaccharides from the culture media the bacteria are grown in to develop a vaccine. The haemophilus influenza type b (HiB), and pneumonococal vaccines are examples of vaccines made using this approach.[5]

In a related approach, we can use the tools of genetic engineering to manufacture a pathogen protein; again exposure to the pathogen protein provides immunity without causing disease. The vaccine for Hepatitis B is based on a protein found on the surface of the virus. We can insert the genes that encode this surface protein into yeast, and use the yeast as a factory to produce the protein for the vaccine.[5]

**History of Vaccines:**

Throughout history vaccination has protected individuals against disease. As early as the seventh century, there are records of Indian Buddhists drinking snake venom to induce immunity (likely through a toxoid effect).[17] In the second millennium, vaccination against smallpox was carried out in central Asia, China and Turkey. People recognized that those who had been exposed to variola (also called cowpox, which produces only mild symptoms) usually did not contract smallpox, an often fatal disease. These two antigens are sufficiently similar that exposure to one protects against the other. In 1721, the idea of variolation against smallpox moved from Turkey to England.[18]

Independently in 1796, Edward Jenner, a country doctor living in England, noted the relationship between smallpox and cowpox. He observed that dairy milkmaids frequently contracted cowpox, which caused lesions similar to that of smallpox. The milkmaids who had cowpox almost never got
smallpox. Jenner carried out an experiment where he injected cowpox pus into a young boy named James Phipps. He later injected Phipps with pus from smallpox sores and noted that Phipps did not contract smallpox. (We will return later to discuss the ethical problems associated with Jenner’s experiment.) Despite the ethical flaws of the experiment, it was a scientific success and Jenner was the first to introduce large scale, systematic immunization against smallpox. While his work ultimately led to the elimination of smallpox from the world, it was not immediately embraced by all. Many people were deeply suspicious of the practice of introducing animal products into their own bodies. During the 1800s, cartoons appeared mocking Jenner and depicting the transformation of the recently vaccinated into sickly cows and fantastic beasts (Figure 8.23).[1]

Despite these concerns, rapid progress followed. In 1885, Louis Pasteur developed the concept of an attenuated vaccine and produced the first vaccine against rabies.[1] In the early 1900s, the concept of toxoid vaccines was developed, leading to vaccines for diphtheria and tetanus.[5] In the 1950s, the tools to maintain cells alive in tissue culture were developed.[19] This scientific advance led to a live attenuated vaccine for polio, a discovery for which Enders, Robbins, and Weller won the Nobel prize. Vaccines for measles, mumps, and rubella were developed in the 1960s.[17]

Table 8.2 shows the dramatic reduction in the incidence of infectious disease in the US following routine vaccination.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peak # of Cases</th>
<th># Cases in 2000</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>206,929 (1921)</td>
<td>2</td>
<td>-99.99</td>
</tr>
<tr>
<td>Measles</td>
<td>894,134 (1941)</td>
<td>63</td>
<td>-99.99</td>
</tr>
<tr>
<td>Mumps</td>
<td>152,209 (1968)</td>
<td>315</td>
<td>-99.80</td>
</tr>
<tr>
<td>Pertussis</td>
<td>265,269 (1952)</td>
<td>6,755</td>
<td>-97.73</td>
</tr>
<tr>
<td>Polio</td>
<td>21,269 (1952)</td>
<td>0</td>
<td>-100</td>
</tr>
<tr>
<td>Rubella</td>
<td>57,686 (1969)</td>
<td>152</td>
<td>-99.84</td>
</tr>
<tr>
<td>Tetanus</td>
<td>1,560 (1923)</td>
<td>26</td>
<td>-98.44</td>
</tr>
<tr>
<td>HiB</td>
<td>~20,000 (1984)</td>
<td>1,212</td>
<td>- 93.14</td>
</tr>
<tr>
<td>Hep B</td>
<td>26,611 (1985)</td>
<td>6,646</td>
<td>-75.03</td>
</tr>
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</table>

Table 8.2 [20]: The incidence of many infectious diseases in the United States was dramatically reduced by vaccines.
For example, there were more than 200,000 cases of diphtheria in the US in 1921, the year of peak incidence. In 2000, only 2 cases of diphtheria were reported in the US; the dramatic reduction in incidence is due to routine childhood immunization. Polio has been eliminated in the US due to vaccination. In 2005, the CDC announced that rubella is no longer a health threat in the US.[21] In 1965, there were 12.5 million cases of rubella in the US. More than 12,000 babies were born deaf, blind or both and 6,200 children were stillborn. In 2004, there were only 9 rubella infections in the US.[22]

Smallpox is one of world’s deadliest diseases, having caused more deaths in history than any other disease. However, smallpox is also the first human disease to be eradicated from the face of the earth by a global immunization campaign. As a result, we no longer routinely immunize against smallpox. The Jenner vaccine was first available in the early 1800s.[1] However, it was difficult to keep the vaccine viable enough to deliver in the developing world. In the 1950s a much more stable, freeze dried vaccine was developed, making it practical to deliver vaccine world wide. In 1959, the Twelfth World Health Assembly set a goal to eradicate smallpox from the globe. However, little progress was made until 1967 when sufficient economic resources were dedicated to vaccinate at least 80% of all populations and to survey for and contain outbreaks. At that time, approximately 10 million cases of smallpox occurred per year. On May 8, 1980, the Certification of Smallpox Eradication declared the world to be smallpox free, and we no longer routinely vaccinate for smallpox.[18]

In the developed world, routine childhood immunization has dramatically reduced the incidence and mortality associated with many diseases. However, the situation is drastically different in the developing world. In 1974, only 5% of the world’s children received 6 vaccines recommended by WHO.[23] At that time, the WHO set a goal to immunize at least 80% of the world’s children against these six diseases by 1990.[17] The program has been a tremendous success, and as of 2004, vaccine coverage has reached nearly 80%. As a result of vaccination, 20 million lives have been saved over the last two decades. Figure 8.24 shows the dramatic reduction in the incidence of reported cases of measles and pertussis during this time period. While these achievements have dramatically reduced child mortality, the 20% of children who do not receive these vaccines account for nearly 1.4 million preventable deaths each year due to pertussis, diphtheria, polio, measles, tetanus and tuberculosis.[24]
With new technologies, it is likely that nearly a dozen vac-
cines will soon be available. Recently licensed new vac-
cines include Gardasil (Merck) to prevent HPV infection and
Prevnar (Wyeth Pharmaceuticals) for pneumococcal dis-
ease. Half of all vaccines have been developed in the last
25 years (about 1/yr, compared to about one every 5 years
before this).[14]

How do we test the effectiveness of new vaccines?
Vaccines are first tested in the laboratory to see if they initi-
ate a response in cell culture or tissue culture systems. If
successful, the vaccine is then tested in animal model sys-
tems. The animal must be susceptible to infection by the
agent against which vaccine is directed and should develop
the same symptoms as humans. Vaccines which are suc-
cessful at preventing disease in animals can then enter hu-
man trials.

We will learn more about the process of testing drugs and
devices in patients later in Chapter 9, but briefly, these trials
have three phases. In Phase I trials, the vaccine is tested in
a small number of volunteers (20-80 persons).[26] Usually
phase I trials are carried out in healthy adults and last a few
months. The goal of phase I trials is to determine the vac-
cine dosages necessary to produce protective levels of im-
munity, as well as to evaluate side effects at these dosages.
Before phase I trials can be carried out, the Food and Drug
Administration (FDA) must approve the vaccine as an Investigational New Drug (IND).

If successful results are obtained in phase I trials (immune protection with minimal side effects), then the vaccine goes into phase II clinical trials. In a phase II trial, a larger number of volunteers (several hundred) are tested, over a period lasting a few months to a few years. Generally, a phase II trial is a controlled study, with some volunteers receiving the vaccine and some receiving a placebo (or existing vaccine). The volunteers are monitored to see if they mount an immune response or contract the disease (vaccine effectiveness) or to see if they develop side effects (vaccine safety).

Finally, vaccines enter phase III clinical trials involving large numbers of volunteers (several hundred to several thousand). These trials last years and are usually carried out as controlled double blind studies, with some volunteers receiving vaccine, some receiving placebo (or existing vaccine). The trial is referred to as double blind because neither patients nor physicians know which was given.

If the vaccine is proven to be safe and effective in phase III clinical trials, the manufacturer can apply to the FDA to sell the vaccine. Licensure by FDA is required before a company can market the vaccine. Generally this requires about a decade. Vaccines must be made following strict manufacturing guidelines and quality control procedures known as current Good Manufacturing Practices (cGMP).

After approval to market a vaccine is given, the FDA continues to monitor vaccine safety in a process known as post-licensure surveillance. Doctors must report adverse reactions after vaccination to the FDA and the Centers for Disease Control and Prevention (CDC). The reporting system is known as the Vaccine Adverse Events Reporting System (VAERS); it receives as many as 12,000 reports per year, of which 2,000 are serious. Most are unrelated to the vaccine, but some can indicate rare but serious side effects that were not observed in phase III clinical trials.

Figure 8.26: By age two children must receive more than 20 shots. Sometimes as many as five shots are required in a single visit to the pediatrician.
How effective are vaccines?

In general, about 1-2 of every 20 people immunized will not have an adequate immune response to a vaccine.[30] Yet, vaccination has largely eliminated many diseases. This occurs because of a phenomenon known as herd immunity. Vaccinated people have antibodies against a pathogen, and as such they are much less likely to transmit that germ to other people. As a result, even people that have not been vaccinated are protected. About 85-95% of the community must be vaccinated to achieve herd immunity.[1]

When herd immunity is lost, outbreaks of once uncommon diseases can occur. In the early 1990s, eastern Europe experienced an outbreak of diphtheria. Universal childhood immunization against diphtheria was introduced throughout the Soviet Union in 1958, and diphtheria incidence dropped and remained at very low levels for more than thirty years. However in the early 1990s, childhood immunization rates fell in the newly independent states of the former Soviet Union. Declining economic conditions, large population migrations and low immunization rates led to a resurgence of the incidence of diphtheria (Figure 8.25).[31] Massive efforts to vaccinate children and adults were required to bring the epidemic back under control.

As vaccines become more commonplace, they have lost their allure in many countries. With the success of widespread immunization, the threat of illness that initially lead to the support of vaccines has diminished. Instead, attention has become increasingly focused on the risks of vaccination. One needs only to Google the terms “vaccine safety” to see the results of this shift in public perception in the United States.[32] In 1954, more Americans knew about the field trial of the Salk polio vaccine than knew the full name of US President Dwight David Eisenhower. At the time, the March of Dimes carried out extensive media campaigns, which increased awareness of the risks polio
Prevention of Infectious Disease

and efforts to develop a vaccine. As a result, most Americans understood the risks of polio and were anxious to be vaccinated.[33] As vaccination has reduced the incidence of infectious disease, groups opposing vaccines have proliferated. An increasingly cynical public sometimes regards information campaigns about the benefits of new vaccines as hype to increase the revenue of pharmaceutical companies. The Internet has facilitated the spread of dissenting views about the risks of vaccination.[32]

For example, some parents and their watchdog groups have raised questions about the link between a rise in autism and the use of the preservative thimerosal in vaccines. A careful series of scientific studies have shown there is no link between thimerosal in vaccines and autism. Even so, the FDA ceased to license thimerosal containing vaccines.[1]

Similar claims about a link between autism and the MMR vaccine have been alleged. In 1998, the journal Lancet published a paper that investigated the link between chronic gastro-intestinal disease and severe developmental regression and autism in a small group of children.[34] The paper noted that most instances occurred after MMR immunization, but the researchers noted they had not yet proved a causal link. Though the paper was appropriately cautious, the lead author of the study, London-based researcher Andrew Wakefield, held a press conference and warned parents that it would be safer if their children received individual vaccinations for measles, mumps and rubella, rather than the combined shot.[32] Fearing a decline in immunization rates, the UK department of health urged parents not to reject MMR vaccinations. Between 1998 and 2004, fueled in part by inflammatory medial coverage, MMR immunization rates declined in Britain to only 80%, falling to just 62% in some areas of London. Two subsequent, larger studies in 1999 showed there was no link between the MMR vaccine and autism. In 2004, collaborators on Wakefield’s paper publicly rejected the link between autism and the MMR vaccine. That same year, Wakefield was accused of having misled Lancet editors by concealing the fact that his research was partially funded by the legal team seeking compensation for parents who believed their children were injured by the MMR vaccine.[32]
Childhood Illness and Vaccines:
Who receives vaccines that have been licensed by the FDA? Generally, recommendations are made by the Center for Disease Control and Prevention (CDC) working in conjunction with expert physician groups regarding when the vaccine should be used and who should receive it. In making recommendations, these experts weigh the risks and benefits of the vaccine, as well as the costs of vaccination.[28] In addition, some vaccinations are required by law. In 1905, the US Supreme Court ruled that the need to protect public health by requiring smallpox vaccination outweighed the individual’s right to privacy.[1] All 50 states have school immunization laws.

These laws provide for exemptions based on medical reasons (50 states), religious reasons (48 states), and philosophical reasons (15 states).[28] Tables 8.3 and 8.4 indicate the CDC recommended childhood and adolescent vaccination schedules in the US, respectively. Children are now routinely immunized against 16 diseases (Figure 8.26).[35]

How Vaccines are Made:
There are substantial scientific and engineering challenges associated with developing new vaccines. For new vaccines to impact public health, we must be able to manufacture hundreds of millions of doses of vaccine. Each and every dose must be safe and effective and equivalent. Because vaccines are given to healthy children and adults, the burden of ensuring that each dose of vaccine is safe is particularly high.[36] Large scale manufacturing of vaccines involves substantial engineering challenges. It requires the ability to take a candidate vaccine developed in a basic research lab and scale up the manufacturing process to make
millions of doses. As we will see, the effective team to do this requires that scientists and bioprocess engineers work closely together; typically a team of at least 10 people is required to lead and coordinate such a complex project.[36] As an example of these challenges, we next consider how the seasonal influenza vaccine is made, and the hurdles that must be overcome in order to produce sufficient vaccine to prevent future influenza pandemics.

**Seasonal Influenza Vaccine:** Influenza is 7th leading cause of death in US. It is the leading cause of death in children aged 1-4 years old, and pneumonia associated with influenza causes 90% of deaths in people over the age of 65.[15] The Advisory Committee on Immunization Practices, a branch of the CDC, recommends an annual influenza vaccine for children between the ages of 6 months and 5 years, pregnant women, people 50 years of age and older, people of any age with certain chronic medical conditions and people who live in nursing homes and other long term care facilities.[37]

The antigenic drift of the influenza virus presents special challenges for developing an influenza vaccine. The number and type of influenza strains circulating among the population varies dramatically from year to year. Generally, it takes 2-4 weeks following vaccination for people to develop protective immunity.[15] Thus, to be effective the influenza vaccine must be available in advance of the peak influenza season. Because of the long lead time to produce millions of doses of the flu vaccine and the time required for vaccinated people to develop immunity, the choice of strain to be used in the vaccine must be made months in advance of flu season, increasing the chances of selecting the wrong strain.

When the influenza vaccine was first developed in the 1940s, it provided protection against only one strain of the influenza virus (monovalent vaccine). In the 1960-1970s, vaccines were developed that protected against two strains (bivalent vaccine), increasing the chances that more people would be protected against circulating strains. Starting in 1978 and continuing to today, three strains are included (trivalent vaccine): two A strains and one B strain. Between 1970 and 2004, the formulation has changed 40
Recent shortages in the availability of the influenza vaccine highlight the engineering challenges associated with producing vaccines. In 2003-2004, two companies manufactured 83 million doses of flu vaccine for the US market. 48 million doses were made by Aventis in the US and an additional 35 million doses were made by Chiron in Liverpool, England. That year, the influenza epidemic started early and the media broadcast many stories describing patients who were hospitalized and died from influenza and subsequent pneumonia. The demand for vaccine exceeded supply and many people could not obtain the vaccine. The shortage of vaccine was even more dramatic the following year. Although more doses were manufactured (Aventis made 55 million doses and Chiron made 48 million doses), there was a manufacturing error at the Chiron plant, and those doses could not be sold.

As a result of these shortages, scientists have begun to examine alternative, more rapid methods of manufacturing the influenza vaccine. The most common influenza vaccine used today is based on an inactivated form of the virus; chicken eggs are used as small bioreactors to grow sufficient quantities of the virus, which is then harvested and inactivated. The current manufacturing process relies largely on technology developed more than 60 years ago.

Once a year, for each hemisphere, experts gather to decide upon the vaccine composition. This is a time consuming step, requiring about seven weeks. Figures 8.27 and 8.28 show the steps in the process of manufacturing the current flu vaccine. In order to produce enough vaccine, approximately 300 million chicken eggs are required; egg production occurs in parallel with strain selection. After the strain of virus has been selected, a form of the actual virus to be grown in eggs must be developed through a process called reassortant preparation. In this process, cells in culture are co-infected with the wild type strain and a strain which has been adapted to grow very efficiently in eggs. The goal is to create a new strain of the virus—one which is capable of producing immunity in people but will grow well in eggs. Eggs are then inoculated with the reassortant preparation in order to produce large amounts of the virus. Fluid containing the virus is then harvested from the chicken eggs, purified using centrifugation and filtration, and inactivated using formalin. This process occurs for each of the three strains. Purified, inactivated virus from each strain is then combined, and packaged into doses of the trivalent influenza vaccine. Inactivated influenza vaccine is stored in the re-
After the vaccine is made, the manufacturer must still carry out phase I, phase II and phase III clinical trials to test the vaccine efficacy and safety. This ENTIRE process (including licensing and safety testing) must be repeated each year. All unused vaccine is discarded. The monovalent concentrates cannot be reused after 12 months. Because the yields of new strains are not known in advance, it is difficult to ensure that the manufacturing process will give sufficient quantities of a new strain.

There are some important advantages of this manufacturing process. Because it has been used for many years it is well tested and understood. The only part that changes from year to year is the structure of the virus to be produced—all other elements in the process can stay the same from year to year. However, because the process is cumbersome and involves long lead times, there are concerns that it will not be possible to produce sufficient vaccine to prevent an influenza pandemic should a virulent new strain of flu emerge. Until recently, egg production was seasonal raising the likelihood that a pandemic might occur at a time when no eggs are ready. Because of this concern, industry has recently changed to a cycle of continuous egg production. Furthermore, because
of concerns that an avian influenza virus could infect populations of chickens that produce eggs to make vaccine, flocks associated with egg production are now under strict biosafety control so they cannot be wiped out.[15]

Updating the manufacturing process may increase the speed with which new vaccine could be produced. Instead of using eggs as bioreactors to grow the influenza virus, it is possible to grow the virus in mammalian cells in culture (Figure 8.29). The process of developing the vaccine is similar, except it is made in the mammalian cells rather than in eggs. The mammalian cells are kept in a bioreactor. The cell line to be used must be able to grow the influenza virus in large numbers, and be suitable for a wide variety of flu strains. Several cell lines which meet these requirements are available and they can be grown in chemically defined, synthetic growth media. The purification and inactivation procedures are similar to those used in egg based systems. An important advantage of mammalian cell culture based systems is that it completely avoids the need to grow eggs from biosecure flocks. In addition, growing virus in this manner may lead to higher initial purity.[41]

Can we switch to systems that use mammalian cell culture? In the event of pandemic influenza, cell-culture based manufacturing of vaccines could provide an advantageous alternative to traditional egg-based systems. At present, there are approximately 1.5 million liters of cell culture capacity in the US. However, this capacity is currently dedicated to the production of other essential drugs and on a very limited basis, vaccines. Given the high cost for construction and validation of a biological production facility (upwards of a billion dollars), it is not economically feasible to have production facilities sitting idle. In the event of an emergency, such as pandemic influenza, cell-culture facilities would need to halt production of other essential drugs in order to accommodate the demand for vaccines. [38]

Another approach to vaccine production is to use recombinant methods to produce a subunit vaccine for influenza. We have seen that the influenza virus has two major antigens on its surface: the hemagglutinin and neuraminidase proteins. The recombinant process involves taking the DNA for HA and inserting it into another type of cell which can then be used as a bioreactor to produce large amounts of the HA protein (Figure 8.30) for use in a vaccine. Yeast are currently used to make the recombinant proteins in the hepatitis B vaccine and in the HPV vaccine.[42]

**Pandemic Influenza Vaccine:** Given the existing egg-based production capacity, let’s examine how long it would take to produce sufficient flu vaccine for global coverage in the event of
a flu pandemic. The current trivalent vaccine contains 15 micrograms of the HA antigen for each strain of the virus. Each year, approximately 300 million doses of the trivalent seasonal vaccine are produced. At current production capacity, then approximately 1 billion doses of monovalent vaccine could be produced. This is enough to immunize only about 1/6 of the world’s population. [14] Table 8.5 shows that it would take almost 5 years to produce sufficient vaccine to provide global coverage.

Unfortunately, it is unlikely that we will have 5 years to produce vaccine in the event of an influenza pandemic. Because antigenic shift is a random occurrence, it is difficult to predict in advance the structure of vaccines that might be protective. To get some idea of how rapidly we might need to manufacture vaccine in the event of a pandemic, we can use simple modeling techniques to predict how rapidly a pandemic might spread throughout the world once a new strain emerges.

In the simplest modeling approach, we divide the population into groups, based on their disease status, and track how the number of people in each group changes over time.[43] Consider a population of $N$ people, divided into the following groups:

$S =$ the number of people susceptible to infection

$E =$ the number of infected people who are not yet contagious

$I =$ the number of infectious people

$R =$ the number of people who have been infected and recovered

When a new strain of flu emerges, the population initially is all susceptible. Over time, people acquire disease, transmit it from person to person, and either die or recover. The decrease in the number of susceptible people as a function of time is given by Equation 8.1.
Chapter 8

Equation 8.1

where \( b \) represents the person to person transmission rate.

The time between the point of infection and the point where a person becomes infectious is known as the incubation period, \( 1/L \). The incubation period is approximately 1.2 days for the influenza virus.[43] The mortality rate due to influenza, \( w \), is estimated to be 0.0005 per day for a virulent pandemic. Thus, the change in the number of infected people versus time is given by Equation 8.2.

\[
\frac{dS}{dt} = -bSI
\]

Equation 8.1

\[
\frac{dE}{dt} = bSI - LE - wE
\]

Equation 8.2

The infectious period, \( 1/g \), is approximately 4.1 days for influenza.[43] We can express the number of people who are infectious with Equation 8.3.

\[
\frac{dI}{dt} = LE - gI - wL
\]

Equation 8.3

Finally, the number of people who have recovered is given by Equation 8.4.
During this period we assume that the birth rate and the death rate due to other causes are the same. We can solve this simple system of differential equations to predict the duration of an influenza pandemic. In order to solve the equations, we need to specify the initial conditions. We assume that the population is at some initial number and begins with one initial infected person; everyone else is uninfected.

The predictions of this simple model indicate that vast majority of cases would occur in the first 200 days of an epidemic.[43] Figure 8.31 shows the predicted number of cases per day following the initial case. The predictions of this very simple model (Figure 8.32a) agree quite well with predictions of much more sophisticated models that take into account factors such as international patterns of population migration (Figure 8.32b right).[43] In addition, these predictions agree well with epidemiologic data from the 1918 Spanish influenza pandemic, and illustrate the scary prospect that in the event of a flu pandemic, vaccine will be primarily available to survivors unless improvements are made in the manufacturing process.[45]

Interestingly, the models predict that it will be far more difficult to control an influenza pandemic than SARS, because a large part of the infectious period associated with influenza occurs before the onset of symptoms. A number of researchers have developed computational tools to understand the most effective approach to prevent an influenza pandemic. Efforts such as border restrictions are unlikely to be effective. Anti-virals must be given within 1-2 days of symptoms to have an effect. Models indicate that the best way to prevent a pandemic is to pre-vaccinate the population. These models show that you need to immunize at least 1/3 of the population if you have a vaccine of perfect efficacy in order to prevent a pandemic.[43] However, with current manufacturing capabilities, making this amount of vaccine will take more than 1 year.[14]

**Economic Challenges:**
Are there economic incentives in place to encourage the type of investment in vaccine research and development and manufacturing processes that is needed to meet global health needs?
Vaccines are made by pharmaceutical companies, and pharmaceutical companies are businesses. The current economic outlook for vaccine products is not encouraging. In 1967, there were 26 companies that made vaccines used in the US; in 2004 there were only five. Since 1998, nine of twelve vaccines recommended for children in the US have been in short supply.[33] As a result, children were delayed in receiving vaccines that they needed, and some children never caught up. It is particularly worrisome that vaccines for seven childhood diseases have only a single manufacturer. What happens if this company experiences a business problem or sudden production failure?

Today the vaccine industry faces major hurdles. The research and development process for a new vaccine is increasingly expensive, lengthy and risky. Companies must build expensive manufacturing facilities, operate within a complex regulatory environment, and deal with a growing anti-immunization movements and a surge in liability litigation. As a result of these factors, it now costs between $110-$800 million to bring a new drug to market, and typically takes more than a decade to bring a vaccine from early development to finished product launch.[26] In 2003, a report from the Institute of Medicine noted with concern the lack of financial incentives for vaccine manufacturers and called for reforms that would encourage investment.[46] In 1998, Warner Lambert stopped making Fluogen vaccine for influenza because of economic considerations and regulatory challenges.[1]

**Figure 8.39:** The time course of HIV/AIDS disease progression.

HIV Testing

We test for the presence of antibodies against HIV using an ELISA (enzyme linked immunosorbent assay). In this procedure, blood is taken from a person who may be infected with HIV. In the lab blood is added to laboratory HIV virus. HIV antibodies from the blood, if present, attach to HIV antigens. Next a chemical that attaches only to antibody/antigen complexes is added. If the solution changes color, the person is making antibodies against HIV. The advantage of ELISA tests is that they are very sensitive, meaning they don’t often miss disease if it is present; the disadvantage of ELISA tests is that they are not very specific, meaning that they sometimes generate a falsely positive result in someone who does not have disease. Thus, a positive ELISA tests requires another test to confirm the presence of disease. The second test is called a Western Blot. Western blots are not as sensitive, but are more specific. Together, these two tests reduce the rate of false positives to 1/250,000.[Chou] If this combination of HIV tests is positive, it indicates that the person is infected with the HIV virus, though he or she may not have AIDS yet. A negative ELISA test indicates either that a person is not infected with HIV, or that the person is infected with HIV but not yet making detectable level of antibodies. In general, it may take 1-3 months (and in rare cases 6-12 months) before the body makes enough antibodies to be detected by an ELISA. [CDC Website]

The ELISA, one of two tests used to identify HIV infection in people. The yellow color indicates the presence of HIV antibodies, a sign of infection with the virus.

Source:[55, 56]
Over the past 50 years, there have been many mergers in the pharmaceutics industry. Companies which previously only made vaccines now make both drugs and vaccines. In these companies vaccine products compete with potential drug products for limited research and development dollars.[33] In general, the market of a drug is much larger—vaccines are only given once, and are often purchased by the federal government. Today, 43% of childhood vaccines are purchased by the private sector. The remaining 57% are purchased through a federal contract which covers children on Medicaid or without health insurance. In 2005, the price for vaccines recommended for children before entering elementary school was $474 if purchased on the federal contract and $782 if purchased privately. Yet, every dollar spent on vaccines, saves $5.80 in direct medical costs![26]

Litigation has also played a role in increasing the costs of vaccines in the US. In the 1970s and 1980s a series of personal injury lawsuits claiming that the pertussis vaccine resulted in complications such as sudden infant death syndrome and mental retardation were filed. Although scientific studies showed there was no link between the vaccine and these adverse events, due to litigation the cost of the pertussis vaccine increased from 17 cents per dose to $11 per dose.[33] To stem this trend, in 1986, the National Vaccine Injury Compensation Program (VICP) was established in the US. The program is funded by a 75 cent tax on each dose of vaccine. Litigants claiming to have been injured by a vaccine must first file a claim through this program. Some injuries are automatically eligible for compensation with no need to prove that the vaccine caused the injury. If the injury is outside the rule, the claimant must prove that the vaccine was the cause of injury. Claimants are free to either accept or reject the decision and award of the VICP. As a condition of accepting an award from the VICP, claimants agree not to pursue further legal action against the vaccine manufacturer. The VICP has paid about $600 million for injuries caused by vaccines administered between 1988 and 2004. Only a small fraction of these funds (2%) were used to cover lawyer’s fees; the rest went to the claimant.[26]

**Challenges of Vaccination in Developing Countries:**
Developing countries now wait an average of 20 years between when a vaccine is licensed in industrialized countries and when it is available for their own populations.[47] Economic, infrastructural, and scientific hurdles all contribute to this long delay.

Vaccines are complex biological substances; they can lose their potency over time. They are more likely to lose potency if exposed to temperatures which are too cold or too warm; some vaccines are also sensitive to exposure to ultraviolet light. This loss of potency is permanent and irreversible. For most vaccines, it is impossible to tell if they have lost potency simply by looking at them (Figure 8.33).[39] If individuals are vaccinated with vaccine that has been damaged in this way, they will not have the desired immune response.

The cold chain is a system that has been developed to ensure that vaccines remain potent as they make the trip from manufacturer to the patient being immunized. The cold chain has three main components—transport and storage equipment, trained personnel, and efficient management procedures. As shown in Figure 8.34, the cold chain begins with the refrigerator at the manufacturing plant, extends through transfer of vaccine to the distributor, then to the provider’s office and ends with administration of the vaccine. It is essential to maintain proper temperature at each step in the process. It has been estimated that 17-37% of providers expose vaccines to improper storage temperatures.[39]

Maintaining the cold chain is a challenge in developing countries, where a lack of infrastructure can make it difficult to maintain proper storage temperatures. When workers suspect that a container of vaccine has not been properly transported or stored, they must throw it away rather than risk using inactive vaccine. A number of tools have been developed to monitor the temperature history of vaccines during the transport and storage processes. In 1996, vaccine vial monitors (VVM) were developed based on technology originally devised for use in the food industry (Figure 8.35). VVMs are small indicators adhered to the tops of vaccine vials; the inner square of the VVM is chemically active and changes color irreversibly with exposure to heat. VVMs can be manufactured for a variety of heat-exposure specifications. Since March 1996, all oral polio vaccine supplied through UNICEF carry VVMs, adding only pennies to the cost of a vial. As of January 2001, all vaccines supplied by UNICEF are required to have VVMs. More than 1 billion VVMs have been delivered to developing countries, and 16 of 25 UN pre-qualified vaccine suppliers include VVMs on their products. The use of VVMs has led to a significant reduction in vaccine wastage because there is an accurate record of their temperature history.[48] Freeze watch indicators (Figure 8.36)
have been developed to monitor whether vaccines have been exposed to temperatures below 0° C. The freeze watch indicator consists of a small vial of red liquid contained in a plastic casing. If exposed to temp below 0° C for more than 1 hour, expansion of the liquid causes the vial to burst, releasing the red liquid.[49]

Most vaccines must be given by injection. This is a particular challenge in developing countries, where health care workers may not have access to an adequate supply of sterile needles. As a result, disposable syringes are often saved and reused. It has been estimated that over 50% of injections given in developing countries follow unsafe injection practices, which can lead to the spread of blood borne diseases.[50] A simple technological solution is now in place to address this challenge. The BD SoloShot™ auto-disable syringe (Figure 8.37) is designed so that when the syringe is filled to a preset level, the plunger stops and can’t be pulled back further ensuring that the correct amount of vaccine is delivered. After one use, the plunger automatically locks so that it can’t be reused. The BD SoloShot™ syringe is manufactured and marketed by Becton, Dickinson and Company.[51] The price of the auto-disable syringe is rapidly dropping and is within 1¢ of disposable syringes. Since its commercial introduction in 1992, more than 2.5 billion immunizations have been delivered using BD SoloShot™ syringes in more than 40 countries in Africa, Asia, Eastern Europe, and Latin America. UNICEF provides only auto-disable syringes to countries that request disposable syringes.[50]

An alternative approach is to develop needle free methods to deliver vaccines. In developed countries, jet injector guns are used to deliver vaccines without needles. These devices rely on a liquid stream at high pressure that is used to penetrate the skin.[49] The jet injector was initially developed for use in mass injection campaigns and could immunize between 600 and 1000 people per hour. From the 1950s to the 1980s they were widely used in US school immunization campaigns and throughout the developing world. However, their use was discontinued in the 1980s, when it was recognized that the multi-use jet injectors had a small risk of transmitting blood borne pathogens from one person to another. Recently, single use jet injectors, such as Biojector2000 (Figure 8.38) have been developed. However, they are currently too expensive for developing countries.[52]

The Global Alliance for Vaccines and Immunization (Gavi) is a partnership between many public and private organizations—including UNICEF, the WHO, the Bill and Melinda Gates Foundation, members of the vaccine industry, and NGOs. Gavi
was formed in 1999 to address the long delay between vaccine availability in industrialized countries and developing countries. Over 5 years, GAVI has committed more than $1.5 billion to improve access to vaccines in more than 73 countries (Table 8.6). Scientific advances that would help make more vaccines available in developing countries include the development of temperature stable vaccines, development of vaccines that required less than three doses to immunize, and the development of needle free methods to administer vaccines.

Despite the truly remarkable advances in public health as a result of vaccines, there are still many infectious diseases for which no vaccine exists. The big three challenges of most importance to developing countries include vaccines to prevent HIV, malaria and tuberculosis. In the last section of Chapter 8, we examine the obstacles which stand in the way of developing a vaccine for HIV.

Designing a New Vaccine: HIV

To understand the challenges involved in developing an HIV vaccine, we must first consider the pathophysiology of HIV/AIDS in more detail. An HIV infection begins when virus is deposited on a mucosal surface. An initial acute infection can sometimes produce mono-like symptoms. Viral dissemination follows, and the patient will exhibit an HIV-specific immune response. As the virus replicates, it destroys an important
component of the immune system - a special kind of T lymphocyte called the CD4+ lymphocytes. The rate of progression of the disease is strongly correlated with viral load. Following initial infection, patients typically experience a long latent period with no clinical symptoms. Eventually, as more and more lymphocytes are destroyed, patients develop Acquired Immunodeficiency Syndrome (AIDS) (Figure 8.39). AIDS is characterized by immunologic dysregulation, accompanied by many opportunistic infections and cancers. The risk of opportunistic infection is correlated inversely with the number of CD4+ lymphocytes. Left untreated, the average patient with AIDS dies in 1-3 years.[54] HIV infection is identified by measuring whether a person is producing antibodies against the HIV virus.

The virus that causes HIV and AIDS was first discovered by Robert Gallo in 1984. At that time, Margaret Heckler, then US Secretary of Health Education and Welfare, predicted that an HIV vaccine would be developed within 2 years. Thirteen years later, in 1997, President Clinton declared that, "an HIV vaccine will be developed in a decade's time." In 2003, President Bush asked congress to appropriate $15 billion to combat the spread of HIV in Africa and the Caribbean, yet there is still no vaccine available to prevent HIV.[57]

There are many reasons that a vaccine has proven so difficult to develop. HIV represents a unique challenge; our bodies can eliminate most acute viral infections. In contrast, our natural immune response does not destroy HIV. In fact, HIV infection results in the production of large amounts of virus, even in the presence of killer T cells and antibody. In developing a vaccine, we are faced with the challenge of trying to elicit an immune response that does not exist in nature. Therefore, we don’t know exactly what type of immune response a vaccine should develop.[58]

How does HIV outwit the immune system so successfully? As HIV replicates inside host cells, it frequently undergoes mutation. Many researchers believe that it is this continuous mutation of the HIV virus that enables it to escape destruction by the immune system. A high mutation rate increases the probability that a new form of the virus will emerge with a genetic advantage that enables it to survive. Figure 8.40 illustrates the ways in which the HIV virus can undergo mutation. As HIV replicates, it uses an enzyme called reverse transcriptase to copy its RNA into double stranded DNA. This DNA is inserted into the host chromosome, where it then directs production of more viral proteins that ultimately assemble into new viral particles. The HIV reverse transcriptase does not proofread this reproduction process, and on average each time the enzyme copies RNA into DNA, the new DNA differs at one base site.[59] HIV
May 29, 2007

He’s a 7-year-old little boy, who like every child I met yesterday, is HIV+, though he currently does not have TB.

I walked into Dr. John’s exam room and sat down in the third chair (where the translator normally sits, but A’s mother speaks English) next to A. He stretched out his hand to shake mine. I shook his hand and he muttered something at me and eventually I made out “my name is A.” He held my hand patiently while I tried several times to sound out his name before getting it right. He then looked at me expectantly and it occurred to me that he was expecting my name. I told him and it took him 3 times to say “Kim” correctly. He then released my hand and went back to listening to Dr. John discussing his health.

Dr. John was telling his mother that A is doing very well. His KS (Kaposi Sarcoma, a malignant type of cancer often found in HIV+ patients, especially in India, the Mid-east and sub-Saharan Africa) is doing well. He is also responding well to ARV therapy. Dr. John told his mother that she should start slowly explaining to A why he comes to the clinic and why he takes so many pills. He will probably soon start question why he should take his medicine if he doesn’t understand. She said that she would tell him one day soon.

His prognosis is so bad, but he is a very sweet boy. It breaks my heart a little.
is the most variable virus known.[60] Additionally, if two genetically distinct forms of the HIV virus with different genetic sequences infect the same cell, DNA from both can integrate into the host genome and produce viral RNA. When new viral particles are packaged, RNA from the different parent viruses can combine to give rise to forms of HIV with entirely new genomes. HIV replicates at a very high rate, so the odds are high that useful mutations will occur over time. Over a 10 year period, thousands of generations of viral reproduction have occurred; during that 10 year period, the virus can undergo as much genetic change as humans would undergo in millions of years![60]

As a result of this high rate of mutation, there are many forms of HIV which a vaccine must provide protection against. The two major types of HIV are HIV-1 and HIV-2. HIV-1 causes a more serious form of the disease and is responsible for the majority of HIV disease throughout the world. There are three main groups of HIV-1; more than 90% of HIV-1 disease is caused by group M HIV-1.[61] Within this group, there are genetically distinct individual strains, called clades (Figure 8.41). It is thought that each strain may require a different vaccine.[62]

Finally, development of a vaccine is complicated because there are many routes of transmission for HIV, including sexual contact and contact with contaminated blood. HIV can be transmitted by contact with virus alone or by contact with cells infected with the virus. Recall that cell-free virus is recognized and eliminated by antibodies, while cells infected with virus are recognized and eliminated by cell-mediated immunity. Thus an HIV vaccine must generate both cell-mediated immunity and antibody-mediated immunity.[58]

What are the design goals for an HIV vaccine? A successful vaccine must produce both antibody mediated immunity and cell mediated immunity against multiple forms of the virus. To develop antibody mediated immunity, the immune system must see virus or viral debris. To produce cell mediated immunity, HIV viral proteins must be presented to immune system on MHC receptors. We have seen three strategies for developing vaccines thus far: inactivated organism vaccines, subunit vaccines and vaccines based on live, attenuated pathogens.

Non-Infectious HIV Vaccine Strategies: A non-infectious vaccine, made using killed virus or a viral subunit, will only stimulate antibody mediated immunity, and thus will not meet the design goal. Animal trials with inactivated whole virus have shown only antibody mediated immunity to a small number of HIV viral subtypes.[63] Similarly, trials of viral subunit HIV vaccines have shown modest antibody mediated immunity effective against a limited number of HIV strains. One type of sub-
unit vaccine has advanced to phase III clinical trials; this vaccine is based on the gp120 protein found in the envelope of the HIV virus. The gp120 protein is needed for HIV to enter cells. Researchers theorized that if patients could make antibody against this protein it could prevent free virus from infecting the patient’s cells. In animal models and phase I clinical trials, it has been shown that this vaccine does elicit production of antibodies against gp120. The antibodies produced neutralized HIV in a test tube. But they only recognized strains of HIV similar to those used to generate the vaccine.[58] As we have seen, the HIV virus is notoriously susceptible to mutation, and these mutations change the structure of the gp120 protein over time so that the antibody may no longer be effective against it.

Despite poor results in animal trials, gp120 subunit vaccines have progressed to human clinical trials. The company Vaxgen developed a subunit vaccine based on the gp120 protein from two clades of HIV-1; the vaccine (called AIDSvax) entered phase III clinical trials in 1998; results were announced in 2003. More than 5,000 volunteers participated in the randomized, double blind, placebo controlled trial. At the beginning of the trial all were HIV negative. 3330 volunteers received AIDSvax, and the remainder received the placebo. After three years, researcher compared the number of HIV infections for those receiving the vaccine and the placebo. 5.7% of those receiving the vaccine developed HIV, whereas 5.8% receiving placebo developed HIV. The difference was not statistically different. Most researchers believe that the effectiveness of this vaccine in preventing HIV infection was limited because it does not induce cellular immunity and provides antibodies against a limited number of HIV strains.

Researchers and public health workers have expressed concern that a vaccine with limited efficacy could actually increase the rate of new HIV infections. They fear that people who receive the vaccine will engage in riskier behaviors if they believe they are protected by a vaccine.[62] Such behavioral changes could possibly negate the benefit of the vaccine with limited efficacy. It is currently not known whether vaccines that do not prevent infection will delay disease progression in infected individuals. This could be an important benefit, especially in parts of the world where access to HAART is limited.[59] Furthermore, it is not known whether such vaccines could reduce viral loads in infected individuals, and this could help curb the spread of disease.

**Live Attenuated HIV Vaccine Strategies:** As we saw earlier, advantage of vaccines that are based on live attenuated forms of a pathogen is that they stimulate both antibody and cell-mediated immunity. Vaccines made using this approach stimu-
lates both B cells and killer T cells and this approach is the most likely to stimulate the necessary immune response. However, because the HIV virus mutates so rapidly, this approach presents unique potential dangers. Because the HIV virus mutates constantly, there is a chance that the attenuated form of the virus used in a vaccine could undergo a mutation that restores its strength. If this occurs, the consequences would obviously be devastating for the person receiving the vaccine. Vaccines based on a live attenuated form of the simian immunodeficiency virus (SIV) have been tested in macaque monkeys. SIV infects monkeys and is closely related to HIV. The vaccine successfully protected the animals when they were exposed to SIV. However, many of the animals vaccinated progressed to AIDS-like symptoms, even when not exposed to the wild type virus although more slowly than those infected with unaltered virus.[58]

Thus, new vaccine strategies are required to develop vaccines to prevent HIV infection. In the remainder of this section, we consider several new approaches under investigation.

**DNA Vaccines:** An interesting new approach to develop vaccines that stimulate both antibody and cell-mediated immunity is to directly inject DNA that codes for viral protein into a patient. This leads the host cells to produce the protein that the DNA codes for; it is processed and loaded onto the MHC receptors and stimulates cell-mediated immunity, without any danger of causing infection. DNA vaccination approaches have shown very successful results in animal trials, generating a strong cell-mediated immune response.[57] Somewhat less successful results have been reported in human trials, where much larger quantities of DNA must be injected to generate immune response. While, many DNA based vaccines are currently in clinical trials, there are also concerns that scientists will not be able to identify a single protein that will elicit immune response against many HIV strains.[62]

**Carrier Vaccines:** The strength of the immune response elicited by a DNA vaccine can be strengthened by using a virus or bacterium that does not cause disease to carry the viral genes of interest to the host cell. Again, protein is produced by the host cells and loaded onto MHC receptors where it stimulates cell-mediated immunity. This approach stimulates both humoral and cell mediated immunity without the danger of real infection. However, immunocompromised individuals can become ill from the carrier. A limitation of the approach is that the carrier must be one that individuals are not already immune to. [62] For the same reasons, booster vaccines cannot be made with the same carrier.
Rural Village Outreach: June 15, 2007

Christina

Lesotho

We went to a rural village where our mentor, Dr. Dudley, had promised the family of a little girl that had passed away that she would return and give back their records, etc. We got there and were greeted by the grandmother who was taking care of the little girl and seemed to be the one in charge around that small corner of the village. She had gathered other children to be tested for HIV, and soon, her little single room Basotho (what they call people or things that are from Lesotho) hut became a testing center for a few different families that came by. A social worker with us performed his first pre-testing counseling, HIV tests, and post-testing counseling for each person tested, and it was interesting to see the reactions to his explanations and what the people being tested were and weren’t comfortable discussing related to HIV and its transmission. I could not believe this was my first time seeing an HIV test kit.

Sophie and I stepped outside for a moment to get some fresh air and see all the children that had gathered. They were all friendly and as soon as the camera came out, the requests for their picture to be taken did not end. They were especially interested in seeing the shots on the screen after they were taken. A few of the older girls spoke English well and one of them was telling me about her interest in school, science, social studies, and traveling. She said she wants to travel to so many countries in Africa and beyond and she hopes to become a nurse and treat others around the world. I encouraged her to return to Lesotho, of course, and kept asking her about her future schooling. She then surprised me with the fact that she cannot pay for high school, so next year may be her last year of school. I could not believe the fees for high school were so high and I really got aggravated by this. I am almost certain it is to keep too many kids from qualifying for university since the govern-
ment provides college scholarships for most students who make it to that point.

The ride to and from the village was really beautiful and it has been so nice not being in a busy city or hectic area. Back at the clinic, we were having lunch and some of the women on the staff at the reception and in social work/counseling started talking about handouts and food assistance and were adamantly against it. They were explaining to us that a mother came in earlier today and started crying when her child tested negative for HIV because that meant she would not get the food supplements the clinic gives to patients on medication. I was so amazed and terrified by this thought. The staff member went on to explain the extreme economic and social problems created by WFP, they call it (World Food Program, I think) and how it has decreased productivity since people have this food source to turn to. She talked about the amount of excess WFP food in villages that she has seen at funerals and other village events, and how terrible it is that the country is being destroyed by foreign aid. I was completely frustrated at the thought.

I feel settled and like I am learning so much each day. I enjoy the people around me and have had an interesting time learning Sesotho words/phrases today. Hopefully I can try out some of my introductions tomorrow.
Promising results have been observed using what is known as a prime/boost strategy. In this approach, a prime vaccine is first given using a carrier to stimulate cell-mediated immunity. This is followed by a boost vaccine using a subunit vaccine to further stimulate antibody mediated immunity. Phase III clinical trials of such a strategy began in 2003 using a canarypox vector to deliver HIV-1 genes that code for several proteins, followed by a boost with the AIDSVAX gp120 subunit vaccine.[59]

Figure 8.43 illustrates another prime/boost approach under investigation is to first deliver naked DNA, and follow with a booster vaccine using a carrier to carry the genes that encode the same protein. Merck is developing such an approach targeted to a core HIV protein called Gag. Researchers believe that this approach may stimulate cellular immunity more effectively.[62] A vaccine based on such a strategy entered phase II trials in September 2005. [66]

Table 8.7 gives an overview of the HIV vaccines currently in clinical trials as of June, 2006. Updates can be found at:

http://www.iavireport.org/specials/
OngoingTrialsofPreventiveHIVVaccines.pdf

Clearly, developing a new HIV vaccine will rely on the contributions of thousands of volunteers who are willing to participate in clinical trials. Scientific and medical progress rely on the sacrifices that healthy people are willing to make for the sake of research—and these risks are real. For example, one risk of participating in an HIV vaccine trial is that it may cause future HIV tests to be positive. This is because rapid tests measure antibodies against HIV and the vaccine may cause people to produce antibodies. Thus, a person participating in a vaccine trial may test positive for HIV, even though they do not have HIV. False positive HIV test results can lead to social stigma. People with positive HIV tests are not allowed to donate blood. Such results can also interfere with the ability of people to get health insurance, obtain employment, or to travel to some foreign countries. Society has an obligation to provide protection for these volunteers. For example, we can pass laws to require insurance companies to change their screening procedures. If an applicant tests positive on an HIV rapid test, they can be required to administer a more sensitive and specific Western blot to discern between false positive rapid test and true HIV infections.[47]

While many volunteers willingly participate in clinical trials, public mistrust has made it more difficult to carry out large trials of candidate HIV vaccines. There is limited public knowledge about HIV vaccine research, and many people harbor suspicion
Prevention of Infectious Disease

that HIV vaccine research is not being carried out for the greater good. The National Institutes of Health conducted a survey of public knowledge and attitudes regarding HIV vaccine research in 2001. Nearly half (46%) of the population surveyed strongly agreed, somewhat agreed or did not know when asked if there was already a vaccine for HIV that was being kept a secret. An even higher proportion of minorities (72% of African American and 49% of Latinos) answered this way. When asked if HIV vaccines being tested could give a person HIV, 69% of the general population strongly agreed, somewhat agreed or did not know. In Chapter 9, we consider the ethical dilemmas that arise in research involving human subjects and we outline the framework of requirements that we have put in place to ensure that such research provides an appropriate balance between the risks and benefits of research.
Rabid dogs and AIDS parades: June 6, 2007

Tessa

I’ve been meaning to blog about the impressive awareness about HIV/AIDS. I have no idea the extent of HIV/AIDS education (i.e., how much Swazis know about how HIV/AIDS is spread and why adherence to treatment is important) but everyone knows it’s a problem. Last weekend, when Dave and I were picking up some fruit from the market (where grandmothers, also called “gogos,” sell produce), we were briefly impeded by a parade of young adolescents holding up signs with various slogans: “No balloon; No party!” and other similar phrases. Two of my favorite campaigns are “I love you, positive or negative” and “I’m over it.” There is a huge stigma against people with HIV here, and the “positive or negative” campaign is geared toward that problem. Even the condoms say, “I love you, positive or negative.” (I only know that because there are condoms in random places around the clinic.) The “I’m over it campaign,” is pretty entertaining. There are billboards in Mbabane that have virtual text message conversations that go something like this: “My wife’s at work. Wanna cum work on me?” “No. Thanks. I’m over it.” There are several different ones. That’s the one that really stuck in my mind though. They’re pretty funny. I think the awareness is restricted mostly to the cities though. My guess is that in rural areas, people probably know a lot less. That reminds me…education here is not free. Many people can’t afford to send their children to school. I was actually talking to my friend Treasure (she works in administration here at the Baylor clinic) about her education experience. She was very lucky and had been sponsored by a woman in California since she was three. The mystery woman paid for all the school fees, uniforms, books, etc. Then Treasure did well enough on her exams to get a scholarship for a university.
Bioengineering and Global Health Project

Project Task 4: Define the problem that your design will address.

In this task, you will likely need to be much more specific about the particular problem you are trying to solve. For example, you may have identified the need for better treatments for tuberculosis in Project Tasks 1-3. In this task, you may want to consider the particular problem of developing a treatment for tuberculosis that increases patient compliance. Turn in a one-page summary of the specific health need that your design will address.

Chapter 8

1. The immune system.
   a. What is an antibody? Describe its structure and its function in the immune system.
   b. Explain the term "immunologic memory."
   c. Describe the cellular-level processes that enable the adaptive immune system to have immunologic memory.

2. When you get a splinter in your toe, the area can become red, hot, swollen and ooze pus. Describe the specific causes of each of these symptoms.

3. Name the 3 general types of immunity and give an example of each.

4. Most common anti-HIV drugs work by inhibiting key steps in viral uptake and reproduction.
   a. Make a drawing which shows the major steps that occur when HIV infects a CD4+ lymphocyte. Indicate on your figure where in the viral life cycle the following classes of drugs act: (1) fusion inhibitors, (2) reverse transcriptase inhibitors and (3) protease inhibitors.
   b. Beginning in the mid-1990s, an increasing number of HIV-infected individuals began a drug regime called highly active antiretroviral therapy (HAART), a combination of three or more anti-HIV drugs taken at the same time. Why is taking a combination of drugs, each targeted against a different aspect of the viral life cycle, so much more effective than taking a single drug?

5. When a TB skin test is performed, a small amount of harmless TB antigen is injected under the skin. The patient monitors for redness and swelling at the site of injection. If a patient has been previously exposed to TB, but does not currently have an active TB infection will redness and swelling be observed? Why or why not?

6. Oh no! You return to Student Health two days after receiving a routine PPD skin test. You have a red bump on your forearm that measures 12 mm in diameter. Every year up until now, your test had been negative.
   a. How does the PPD skin test work, and why does a red bump form for individuals infected with TB?
   b. Assuming you have no significant health problems, what are the odds that the bacterium will remain in a latent, inactive state for the rest of your life?
   c. The PPD skin test is imperfect. Describe one instance in which the PPD skin test fails by giving a false-negative result, and describe another instance in which the test fails by giving a false-positive result. Why does the test fail in each circumstance?

7. If you are exposed to the varicella virus as a child and have not been vaccinated, you will likely develop chicken pox. If you are exposed again as an adult, you probably will not develop the disease again.
   a. At first exposure, what type of immunity fights off the varicella virus?
b. The varicella vaccine contains a live virus. Is this safe? Why or why not? What is the advantage of this type of vaccine over a vaccine made of a dead virus?

8. A 24-year-old HIV-positive man is hospitalized because he developed pneumonia. The doctor starts the patient on antibiotics and measures the number of CD4 helper T cells in the patient’s blood. The patient has a low CD4 count.

   a. What are two of the three major transmission routes by which this man might have become HIV-positive?
   b. The doctor then performs a test and finds that the man’s serum is positive for antibodies to gp41 and gp120, the HIV envelope glycoproteins. Name and briefly describe this test the doctor ordered.

9. Answer the following questions about pathogens, the immune system, and vaccines.

   a. Check to indicate pathogen type(s) for which each statement applies.

   
<table>
<thead>
<tr>
<th>Trait</th>
<th>Bacteria</th>
<th>Virus</th>
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</thead>
<tbody>
<tr>
<td>Uses host cellular machinery to reproduce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can be killed or inhibited by antibiotics</td>
<td></td>
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<tr>
<td>Short pathogen peptide sequences are displayed in MHC surface receptors.</td>
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<td></td>
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<tr>
<td>Living cells, usually having both a membrane and cell wall</td>
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<tr>
<td>Protein capsid houses nucleic acid core</td>
<td></td>
<td></td>
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<tr>
<td>Can reproduce without a host</td>
<td></td>
<td></td>
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<tr>
<td>Tens of nanometers in size</td>
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</table>

   b. How can T cells identify cells infected with viruses?
   c. Antigen binding to B-cell surface receptors and interaction with activated helper T cells activates B-cells to produce and secrete antibodies. Compare the onset and magnitude of the B-cell response for primary (initial) and secondary (subsequent) exposure to a particular antigen.
   d. Identify the following vaccine types from the descriptions provided. Which one is likely to confer life long immunity?

   ________________: The pathogen is treated with chemicals or irradiated. The early version of the polio vaccine and the rabies vaccine are examples.

   ________________: Mutations have been introduced to the pathogen. This form is used to prevent measles, mumps and rubella.

10. The incidence of many diseases has been reduced by widespread vaccination. However, vaccines are not available for some diseases.

   a. Name three diseases for which vaccines are most critically needed to improve world health.
   b. For one of the diseases you listed in part a, explain the major scientific and economic challenges associated with developing a vaccine.

11. Technologies for vaccine development and delivery are considered among the top ten biotechnologies that may improve health in developing countries. Imagine that you are a member of GAVI
evaluating new vaccination strategies for adoption by the organization. You are asked to choose between an oral live attenuated (Sabin) and an injectable inactivated (Salk) polio vaccine for use in Sub-Saharan Africa. Polio is a viral disease that can produce paralysis. It is passed through fecal-oral transmission. Assume that the two vaccines have equal efficacy in preventing polio infection.

a. What does GAVI stand for?

b. Place the following stages of viral infection and replication in the correct order:
   1. Synthesis of viral proteins
   2. Viral budding or cell lysis
   3. Endocytosis/injection or viral contents
   4. Binding to cell membrane

c. How does a live attenuated vaccine differ from an inactivated vaccine?

d. Which of the following components of the immune system must a vaccine stimulate?
   i. macrophages
   ii. B cells and T cells
   iii. neutrophils
   iv. innate immunity
   v. complement

12. It has been shown that unvaccinated contacts of babies who receive the Sabin Polio vaccine will develop antibodies to the virus, while unvaccinated contacts of babies who receive the Salk Polio vaccine will not.

a. Explain why this might be the case.

b. List two reasons why the Sabin vaccine might be preferable to the Salk for use in Sub-Saharan Africa.

c. What is the main risk of using the Sabin poliovirus in an immunocompromised population?

d. There has been much interest in eliminating poliovirus worldwide. What is the only infectious disease that has been eradicated to date?

e. Discuss why the use of a vaccine led to the eradication of this disease while other diseases for which vaccines exist have not been eradicated.

f. List two properties that are necessary in order for a disease to be eradicable.

g. Which vaccine would you recommend that the GAVI adopt? Name two reasons why.

h. Why has there been so much focus on and investment in vaccination as a strategy in world health?

13. Portions of the following article appeared in the Austin American Statesman on May 10, 2005. Please read the article and answer the following questions.

Questions about pertussis article:

a. The article states that the pertussis vaccine does not protect 15-20% of children who receive it. Discuss how the concept of ‘herd immunity’ will protect these children. What fraction of the population must be vaccinated to achieve ‘herd immunity’?

b. The article describes a new booster vaccine called Boostrix. It states that the new vaccine may be available commercially next month. What process will the FDA use to ensure that the vaccine is safe after it is approved for general use? Why is this process necessary?

c. Pertussis is generally a mild disease in adults and older children. What arguments would you make in support of widespread distribution of the booster vaccine?
Travis County investigating outbreak of whooping cough

County leads state in number of cases; State also could have another bad year for pertussis

By Mary Ann Roser
AMERICAN-STATESMAN STAFF
Tuesday, May 10, 2005

Local health officials are investigating an outbreak of whooping cough as Travis County copes with the bleak distinction of having the state's most reported infections of the highly contagious disease, also known as pertussis, so far this year.

The Austin/Travis County Health and Human Services Department reported 58 confirmed cases of pertussis since Jan. 1, an unusually high number. The county has not had a whooping cough death since 2003. That year, infant Serena King died of the illness, which causes a violent cough followed by a whooping sound.

King was younger than 2 months, the age at which babies get their first pertussis vaccination, when she died. State health officials are awaiting confirmation of a suspected pertussis death this year, but the patient was not from Central Texas, said Rita Espinoza, an epidemiologist at the Department of State Health Services.

Pertussis is on the upswing nationally, and if current trends continue in Texas, 2005 could be one of the worst years since vaccines have been available.

As of April 30, the state had a preliminary count of 269 pertussis cases, compared with 192 during the same period a year earlier, according to the state health department. The worst year for whooping cough since the introduction of vaccines in the 1940s was 2002, when the state reported 1,240 pertussis cases, Espinoza said.

Health officials are worried.

In 2004, the preliminary count was 1,174 whooping cough cases statewide, compared with 670 in 2003. Travis County reported 97 cases in 2004 (the state's count for Travis County was higher, at 125; the two will reconcile the numbers later), and the county had 62 whooping cough cases in 2003, said Dr. Adolfo Valadez, the health authority for the Austin/Travis County department.

"It's a concern all over the state," Espinoza said. "I was just down in the Valley last week, and I was informed of 20 to 25 cases in an area where we usually don't hear of that many. We need to find a way to curb the cycle."

The outbreak is a warning to parents to keep their children's immunizations up-to-date, Valadez said.

It has picked up steam in the past five to six weeks, and most of the cases are in babies younger than a year old and children from ages 10 to 15, Valadez said. Schools in Austin and Pflugerville are seeing sporadic cases, but "no schools have had to be closed," he said. "Quite, honestly, we're looking forward to school ending. That's how it spreads."

Espinoza and Valadez said other factors could be contributing to the uptick in cases in recent years: growing awareness of pertussis, a quicker test to diagnose the illness and waning immunity from the whooping cough vaccine.

The vaccine has been changed to reduce some side effects, which could cause immunity to wear off in less than five to 10 years, Espinoza said. Also, the vaccine is far from foolproof. It does not protect 15 percent to 20 percent of the children who get it, which means adolescents can get pertussis and spread it to young children and babies who are at greatest risk of serious illness.

A week ago, the Food and Drug Administration approved the use of a pertussis booster vaccine, Boostrix, for children from ages of 10 and 18. Valadez said it is expected to be available commercially as early as next month, and he was encouraged that the tool was coming to the public health arsenal.

Now, children are vaccinated for pertussis at 2 months, 4 months, 6 months and between 15 months and 18 months, with a booster between ages 4 and 6, Espinoza said.

Pertussis bacteria live in the nose, mouth and throat and escape into the air when people sneeze, cough and talk. The disease is usually mild in older children and adults but can cause breathing problems, pneumonia and swelling of the brain. It begins like a cold, with a mild fever and cough, which slowly worsens and leads to coughing fits that sometimes end in vomiting.
14. Google the terms:
   - Vaccine and safety
   - Vaccine and dangers

   Do you think the sites that pop up on the two searches contain accurate health information? Why or why not? If you were a pediatrician, what would you tell the parents of your patients who had performed similar searches? A short paragraph is sufficient.

15. You have been asked to write a 500-525 word column on the Avian influenza situation for the BIOE Tribune. Your editor informs you that you must write a critique of the US plan in case of an Avian influenza pandemic. Your critique should include the scientific, economic, and public health aspects of this plan. Other topics, including potential vaccine strategies, may be addressed as well. The CDC website http://www.cdc.gov/flu/avian/ may provide information which will be helpful in completing this assignment. REMEMBER this is for a newspaper so make it compelling and make it interesting, but also make it TRUE!

16. Who sings “The Avian Flu…a three minute summary”? (Hint: She also sings “King of the Rollerama” and “The Great Metric Threat of 79”.)
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