How vaccines work: immune effector mechanisms and designer vaccines

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ABSTRACT
Introduction: Three major advances have led to increase in length and quality of human life: increased food production, improved sanitation and induction of specific adaptive immune responses to infectious agents (vaccination). Which has had the most impact is subject to debate. The number and variety of infections agents and the mechanisms that they have evolved to allow them to colonize humans remained mysterious and confusing until the last 50 years. Since then science has developed complex and largely successful ways to immunize against many of these infections.

Areas covered: Six specific immune defense mechanisms have been identified. neutralization, cytolytic, immune complex, anaphylactic, T-cytotoxicity, and delayed hypersensitivity. The role of each of these immune effector mechanisms in immune responses induced by vaccination against specific infectious agents is the subject of this review.

Expertopinion: In the past development of specific vaccines for infections agents was largely by trial and error. With an understanding of the natural history of an infection and the effective immune response to it, one can select the method of vaccination that will elicit the appropriate immune effector mechanisms (designer vaccines). These may act to prevent infection (prevention) or eliminate an established on ongoing infection (therapeutic).

Literature search: The primary literature source is Pub Med. Secondary source is Wikipedia.

1. Introduction
1.1. Space invaders
The wide variety and abilities of infectious organisms to evade human defense mechanisms is analogous to the invaders of Earth from Space envisioned in movies and comic books. By ‘clever design’ or evolution, we have developed a variety of both specific (adaptive) and nonspecific (natural or innate) means to defend against the myriad of invaders of our space, i.e. our bodies [1]. Unfortunately for us, either the design is imperfect or evolution includes not only development of immune protection for us but also a means for invaders to evade our protective mechanisms.

1.2. Natural immunity
Nonspecific innate or natural immunity mechanisms do not require immunization or specific recognition of invaders. These include chemical and physical barriers, natural killer cells, activated macrophages, and humoral components such as lysozyme, acute phase proteins, complement, natural antibodies, etc [2]. These are not activated by vaccination but may contribute to a more robust adaptive immune response (danger hypothesis [3]).

1.3. Adaptive immunity
The specific adaptive immune system includes a variety of immune effector mechanisms directed by specific regions of receptors (paratopes) on induced immunoglobulin molecules (antibodies) or T cells to epitopes (antigens) on individual infectious agents.

1.4. Immune effector mechanisms
Adaptive immunity works by specific immune effector mechanisms directed to counteract the properties of infectious agents that allow them to survive, proliferate and cause disease. Prior to the publication of the book ‘Clinical Aspects of Immunology’ by Phillip Gell and Robin Coombs in 1963 [4], there was no useful classification of adaptive immune reactions or immune-mediated diseases. There was a long list of in vitro reactions, inflammatory phenomena, and diseases that provided a confusing and overwhelming view of immunology and immunity. Gell and Coombs proffered four immune mechanisms;
- Type I: Atopic or anaphylactic;
- Type II: Cytolytic;
- Type III: Immune complex;
- Type IV: Delayed hypersensitivity (DTH).
This has been expanded by 3 [1]: Neutralization added; DTH has been divided into two: DTH and T-cell cytotoxicity (T-CTL), and Granulomatous reactions added.
Examples of the ‘double-edged sword’ of immune effector mechanisms in protective immunity and in disease are listed in Table 1. Immune effector mechanisms may not only act...
effectively against foreign invaders, but also may cause disease when not appropriately applied or when directed to self (auto-immunity). The objective of this review is to list the specific immune effector mechanisms that are activated and function in defense against infection and after vaccination [5].

1.4.1. Neutralization reactions
The effect of neutralization reactions is to inactivate or block the activity of biologically active molecules. The may be accomplished by two mechanisms: inactivation or receptor blockade. Inactivation by antibody reactions with a biologically active molecule (hormone, enzyme or toxin) usually involves alteration in the tertiary structure so that the biologic agent can no longer function [1]. This is the primary mode of action of vaccines against diphtheria, tetanus, pertussis and cholera toxins. Antibodies may also eliminate antigens by activating macrophages to remove them from the circulation ‘opsonization’. This is believed to be one mode of action of antibodies to Tau protein following immunization of individuals with Alzheimer’s disease (see below). Neutralizing antibodies may also block the interaction between a ligand and receptor so that they cannot bind. To replicate viruses must enter cells and they do this through specific receptor-ligand interactions. Antibodies to the surface ligands on viruses prevent binding to receptors on cells (receptor blockade). Antibodies to receptors on normal cells are responsible for some diseases, such as myasthenia gravis, pernicious anemia, hemophilia, and some types of diabetes. Auto-antibodies to a receptor on thyroid cells actually activate thyroid hormone secretion and cause hyperthyroidism [1];

1.4.2. Cytotoxic reactions
Antibodies to cell surface receptors may activate complement-mediated lysis. This mechanism usually is directed to cells in suspension, such as blood cells. This mechanism is active against bacterial infections, such as streptococcus, staphylococcus and salmonella, and may cause hemolytic anemia, loss of platelets (thrombocytopenia) and loss of white blood cells (agranulocytosis).

1.4.3. Immune complex reactions
Antibody and complement (toxic complex reactions) activate acute inflammatory reactions seen in many bacterial infections resulting in opsonization of bacteria and infiltration and activation of polymorphonuclear cells. This mechanism is responsible for abscess formation in bacterial infections (Streptococcal, Streptococcal, etc.) and, if effective, cure the infection, and if not may lead to irreversible tissue damage and scarring. Activation of the complement system may be shared by at least three mechanisms: Neutralization: removal of molecules by opsonization; Cytolysis; lysis of red blood cells, and Immune Complex: attraction and activation of polymorphonuclear leukocytes by complement components.

Table 1. The ‘double-edged sword’ of immune effector mechanisms.

<table>
<thead>
<tr>
<th>Immune effector mechanism</th>
<th>Protective function</th>
<th>Destructive function</th>
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</thead>
<tbody>
<tr>
<td>Neutralization (inactivation)</td>
<td>Diphtheria, tetanus, cholera Endotoxin neutralization</td>
<td>Insulin resistance, bleeding (blood clotting factors)</td>
</tr>
<tr>
<td>(receptor blockade)</td>
<td>Block viral-cell interactions</td>
<td>Myasthenia gravis, Grave’s disease</td>
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<tr>
<td>Cytotoxic</td>
<td>Bacteriolysis</td>
<td>Anemia, leukopenia</td>
</tr>
<tr>
<td>Immune complex</td>
<td>Acute inflammation, opsonization</td>
<td>Vasculitis, glomerulonephritis, Arthritis</td>
</tr>
<tr>
<td>Atopic</td>
<td>Vasodilation, intestinal parasites</td>
<td>Asthma, anaphylaxis, hay fever</td>
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<tr>
<td>T-cell cytotoxicity</td>
<td>Viral exanthems, influenza</td>
<td>Contact dermatitis, graft rejection</td>
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<tr>
<td>Delayed hypersensitivity</td>
<td>Tuberculosis, leprosy</td>
<td>Auto-allergies, post-vaccinal encephalitis</td>
</tr>
<tr>
<td>Granulomatous</td>
<td>Tuberculosis, helminths</td>
<td>Sarcoïdosis, berylliosis</td>
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</table>

1.4.4. Atopic and anaphylactic reactions
These reactions serve to open blood vessels during inflammation and play a general role in protection against infection by initiating inflammation (vasodilation). For intestinal worm infestations atopic reactions may cause expulsion of the worms (Helminths). Anaphylactic reactions in the lung lead to asthma and in the peripheral blood vessels to generalized vasodilation (shock). In viral lung infections sneezing and coughing may not only result to elimination of virus and virus infected cells, but also contribute to spread of the disease.

1.4.5. T-cell cytotoxicity
Cytotoxic T-cells (T-CTLs) react against viral infected cells and are a mechanism for elimination of virus infections. This is especially important in small pox, measles, papilloma virus, hepatitis virus and polio virus infections as well as influenza, and is the primary immune mechanism in tumor immunity. The classic T-CTL reaction is contact dermatitis (poison ivy) where specific T-CTL attack a foreign antigen attached to skin epithelial cells. This is like the epithelial reaction seen in small pox and measles. Autoimmune diseases due to T-CTL are Hashimoto’s disease (thyroiditis), Chron’s disease, psoriasis, and vitiligo.

1.4.6. Delayed hypersensitivity
DTH is mediated by the activation of monocytes by products of CD4 + T cells reacting with antigen. It is the primary mechanism of defense in tuberculosis, leprosy, syphilis, and candidiasis. Tuberculosis and leprosy infect macrophages. CD4 + T cells react with antigen on the infected cells, activating phagocytosis and digestion of the organisms. DTH is the effector mechanism for demyelinating diseases.

1.4.7. Granulomatous reactions
Granulomatous reactions may be formed after activation of a specific or nonspecific mechanism. They are space occupying collections of monocytes, giant cells and fibrous tissue that form in response to material that cannot be easily broken down and removed by macrophages Granulomas form when DTH is unable to clear an infection. If effective they serve to wall off infected cells or organisms, such as fungi. If immunity
is not effective the granulomas continue to grow and produce disease by occupying space. This is seen in military tuberculosis and in diseases such as berylliosis. Sarcoidosis and rheumatoid nodules. For more details on immune effector mechanisms and how they function in health and disease please see reference [1].

1.4.8. Vaccine design

Vaccines should be designed to stimulate the effector mechanism that is known to be effective from the natural history of each infection [5] and the immune response that is effective against it. The immune effector mechanisms called into play by specific vaccination against the infectious agents are listed Table 2. Effective vaccines may act in two ways: induction of an immune mechanism that will prevent infection or one that will provide for therapy once the infection has occurred. For example, in virus infections prevention is accomplished by blocking the reaction of the infectious agent with the cells that it will infect; therapy is directed to killing already infected cells or organisms that survive outside of cells.

2. Vaccines and immune effector mechanisms

2.1. Toxin neutralization

2.1.1. Diphtheria

The signs and symptoms of diphtheria are caused by a toxin released by infections with Corynebacterium diphtheriae. The bacteria infect the salivary glands in the throat and can cause difficult breathing and swollen lymph nodes. The toxin gene is encoded by a bacteriophage (a virus that infects the bacteria). The toxin enters the cytoplasm of human epithelial cells and inhibits protein synthesis [6]. The toxin is a single polypeptide

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Preventive</th>
<th>Therapeutic</th>
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<tr>
<td><strong>Toxin neutralization</strong></td>
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<tr>
<td>Diphtheria</td>
<td>Toxin inactivation</td>
<td>T-CTL</td>
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<td>Tetanus</td>
<td>Toxin inactivation</td>
<td>Toxin inactivation</td>
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<tr>
<td><strong>Receptor blockade and toxin neutralization</strong></td>
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<tr>
<td>Pertussis</td>
<td>IgA receptor blockade</td>
<td>Toxin inactivation, T-CTL, DTH</td>
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<tr>
<td>Cholera</td>
<td>IgA receptor blockade</td>
<td>Toxin inactivation</td>
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<td>Anthrax</td>
<td>IgA receptor blockade</td>
<td>Toxin inactivation</td>
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<tr>
<td><strong>Receptor blockade and immune complex reaction</strong></td>
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<tr>
<td>H. Influenza</td>
<td>Receptor blockade</td>
<td>Immune complex</td>
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<td>Meningococcus</td>
<td>Receptor blockade</td>
<td>Immune complex (opsonization)</td>
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<tr>
<td>Pneumococcus</td>
<td>Receptor blockade</td>
<td>Immune complex</td>
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<tr>
<td>Streptococcus A</td>
<td>IgA Receptor blockade</td>
<td>Immune complex (opsonization)</td>
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<td><strong>Receptor blockade and T-CTL</strong></td>
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<tr>
<td>Smallpox</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<tr>
<td>Measles</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<tr>
<td>Rubella</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<tr>
<td>Varicella</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<tr>
<td>Influenza</td>
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<td>T-CTL</td>
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<td>Mumps</td>
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<td>T-CTL</td>
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<td>Ebola</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<td>Rotavirus</td>
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<td>HIV</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<td>Rabies</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<td>Hepatitis</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<td>Polio</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<tr>
<td>Chagas disease</td>
<td>Receptor blockade</td>
<td>T-CTL</td>
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<td>Epstein Barr virus</td>
<td>Receptor blockade</td>
<td>T-CTL, DTH</td>
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<td><strong>Receptor blockade and DTH</strong></td>
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<tr>
<td>Yellow fever</td>
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<td>DTH</td>
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<td>Japanese encephalitis</td>
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<td>Dengue</td>
<td>Receptor blockade</td>
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<td>Chikungunya virus</td>
<td>Receptor blockade</td>
<td>DTH</td>
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<td>West Nile</td>
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<td>Zika</td>
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<tr>
<td>Typhoid fever</td>
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<td><strong>Cytolytic</strong></td>
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<tr>
<td>Malaria</td>
<td>Cytotoxic antibody</td>
<td>T-CTL</td>
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<td><strong>Atopic</strong></td>
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<td>Helminths</td>
<td>Atopic</td>
<td>DTH</td>
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<td>Schistosomiasis</td>
<td>Atopic</td>
<td>DTH</td>
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<td><strong>Immune complex</strong></td>
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<tr>
<td>Staphylococcus aureus</td>
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<td>Toxin inactivation</td>
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<td>Lyme disease</td>
<td>Immune complex</td>
<td>DTH</td>
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<td><strong>T-CTL</strong></td>
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<td>Papilloma</td>
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<td>Tuberculosis and leprosy</td>
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<td>Syphilis</td>
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<td>Candidias</td>
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chain of 535 amino acids consisting of two subunits linked by disulfide bridges [7]. One unit ‘B’ binds to the cell surface through epithelial growth factor like receptors and the other unit ‘A’ penetrates the cytoplasm through endocytosis where it inhibits RNA translation and causes death of laryngeal and bronchial cells. Prominent are pseudo-membranes, which are formed from waste products and protein from intoxicated cells. The Schick test developed by Bela Schick in 1925 was used to test for immunity to the toxin. In this test 0.1 ml of toxin is injected into the skin of one arm, and 0.1 ml of inactivated toxin into the other. Individuals with neutralizing antibody will have no reaction at either site; those without antibody will have acute inflammation at the toxin site but not the inactivated toxin site [8]. The vaccine is usually included in trivalent diphtheria, pertussis, tetanus (DPT) immunizations. As with tetanus vaccination, 10-year booster injections are highly recommended. The toxoid is produced by heat treatment or partial denaturation of toxin released in vitro cultures of C. diphtheriae with formalin [9] and absorption to aluminum salts (alum), which increases immunogenicity. The vaccine limits the severity of the disease so that an immune response can eventually eliminate infected cells (T-CTL). Use of the vaccine has resulted in a more than 90% decrease in the number of cases globally [10].

2.1.2. Tetanus

The vaccine for tetanus is an inactivated form of a toxin which causes life-threatening muscle contractions particularly in the jaw and neck muscles (lock Jaw) that interfere with the ability to breath [11]. Tetanus is caused by extremely durable spores of the Clostridium tetani bacteria that live in soil, saliva and manure [12]. The spores enter tissues usually through a cut or wound and convert to the vegetative form in the anaerobic environment of damaged tissue. The toxins are released from the damaged tissue into the body. There are two toxins, tetanolysin and tetanospasmin [13]. Tetanolysin damages viable tissue surrounding the infection and contributes to growth of the vegetative form of the organism. Tetanospasmin is very potent, enters the circulation binds to nerve terminals and produces the muscle contractions characteristic of the clinical symptoms. In 2015 there were about 209,000 infections and 60,000 deaths globally; down from 356,000 deaths in 1990 [14]. The tetanus vaccine is made of inactivated tetanospasmin toxin (toxoid) that has immunizing epitopes but no biologic activity. The toxoid is made by treating the toxin with chemicals, such as formalde-hyde [12]. The amount of antibody produced is measured by international units (greater than 0.01 IU) is considered protective. The international unit is determined by enzyme-linked immune-absorbent assay (ELISA) and compared to the World Health Organization (WHO) reference sera. This level is usually greatly exceeded after the first immunization but decreases with time, so booster immunizations are recommended every 10 years. The vaccine is essentially 100% effective [12] and almost all cases of tetanus occur in people who are unimmunized or have not had a booster in the preceding 10 years [14]. The tetanus toxoid is usually included in a form called DTaP which includes diphtheria and pertussis vaccine [15]. The mechanism of action is neutralization by which antibody reacting with the toxin modifies it so that it cannot bind to nerve termini. Since the organisms are in dead tissue and do not infect the body, an immune response to C. tetani itself is not required for protection [1].

2.2. Receptor blockade and toxin neutralization

2.2.1. Pertussis

Bordetella pertussis is a Gram-negative, aerobic coccobacillus which infects the ciliated epithelium of the larynx and pulmonary bronchi leading to cough and difficult breathing (whooping cough) [16]. During infection it penetrates the epithelium and macrophages through reaction of a filamentous hemagglutinin with receptors on cells and multiplies within cells where it produces at least four toxins [17]. The toxins inflict local mucosal damage with heavy exudates characteristic of the infection. This sets the stage for multiple possible immune-protective mechanisms [16]. First innate immune mechanisms (dendritic cells, NK cells, macrophages and polymorphonuclear cells) may limit the infection, but eventually specific active immunity is much more effective. This is induced by the five-component pertussis vaccine which includes acellular pertussis antigens, detoxified pertussis toxin (toxoid), filamentous hemagglutinin, pertactin and fimbriae types 2 and 3, absorbed to alum [18]. Specific active immunity includes antibodies to pertussis toxins, antibody to filamentous hemagglutinin, T-lytotoxic cells and delayed hypersensitivity (T-cell-mediated macrophage activation) [16]. Thus, the multi-directed immune response blocks cell entry, inhibits toxins, and directs T-lytotoxic cells to infected epithelial cells and DTH to infected macrophages. The widely used pertussis vaccines induce good antibody responses, but weak cellular responses. Recently pertussis vaccines have been modified to induce cell-mediated immunity by replacing alum with alternative adjuvants, such as toll-like receptor. It has also been suggested that pertussis vaccination might be more effective if given separately from diphtheria and tetanus immunizations [16].

2.2.2. Cholera

Cholera is an acute diarrhea caused by Gram-negative bacterium Vibrio cholerae. Cholera was described in India in the fifth century BC and spread in a world-wide epidemic in 1817. There have been 7 epidemics since then, the last in 1961. The WHO estimates that there are up to 5 million cases per year [19]. It is acquired orally from contaminated water and can be prevented by appropriate sanitation. In the 1850s, a London physician, John Snow, demonstrated that the source of a cholera epidemic was contaminated well water, and was able to halt an epidemic. It is now endemic in many areas of Asia and Africa and in the past 10 years devastating epidemics of cholera have broken out in Angola, Ethiopia, Zimbabwe, Pakistan, Somalia, Vietnam and Haiti. V. cholerae has more than 200 serotypes based on the lipopolysaccharide O Antigen; only types O1 and O 139 cause epidemics. Originally susceptible to most antibiotics, now antibiotic resistant strains are more common. After ingestion most organisms are killed by gastric acid, but surviving organisms colonize the small intestine and release cholera toxin, which enters intestinal epithelial cells and greatly increases chloride secretion.
leading to secretory diarrhea. Rehydration is an effective treatment and with proper care the mortality can be reduced to less than 2% [20]. Safe drinking water is the key to prevention. Effective vaccination is directed to mucosal immunity. This is accomplished by oral administration of killed bacteria which was introduced in the 1990s. A single dose oral vaccine is now recommended for travelers and millions of doses have been given [19]. Two oral vaccines are licensed. The Dukoral® monovalent vaccine from Sweden contains formalin, heat killed whole cells of *Vibrio Cholera* O1 and a recombinant cholera toxin B subunit. The Sanchol/mORCVAX® vaccine from Vietnam combines the O1 and O139 serogroups [19]. The vaccines work by blocking the binding of the polysaccharide on the bacteria to epithelial receptors and inhibition of the protein exotoxin, which is encoded within the genome of a filamentous bacteriophage, CTXϕ [20]. These vaccines provide about 85% protection for 6 months and 50%-60% for the first year (WHO). An injectable vaccine is also available, but rarely used.

### 2.2.3. Anthrax

Anthrax is a disease of hoofed animals (sheep, cattle, etc.) caused by *Bacillus anthracis*. Humans are infected by contact with infected animals or their products. The manifestations of the disease are determined by the route of contact, cutaneous (skin ulcers), inhalation (acute pneumonia), gastrointestinal (fever, vomiting, nausea) and inoculation (abscess) [21]. Death is common (20% in cutaneous and over 50% after inhalation). The anthrax bacillus also exists in a spore form where it is highly resilient to changes in environment or harsh chemical treatment. The characteristics of *B. anthracis* make it an ideal biological weapon [22]. Postal distribution of spores in 2001 led to antibiotic treatment of more than 30,000 potentially exposed individuals. The pathogenesis of anthrax in determined by the presence of plasmids that encode a tripartite anthrax toxin (AT) complex [23]: Lethal factor (LF, a metalloprotease), edema factor (EF, an adenylate cyclase) and protective antigen (PA-a ligand that transports LR and EF into the cell). EF inhibits phagocytosis and causes edema; LF causes toxic shock and death. A live-attenuated form of *B. anthracis* introduced in 1881 by Louis Pasteur (23A), was refined, and is now used in China and Russia. This has been discontinued in the western-world due to toxic effects such as skin necrosis at the injection site [23]. Now refined culture supernatant from an attenuated strain that immunizes against PA, known as AVA is used [24]. This stimulates production of antibody to PA, and blocks toxin endocytosis. After three doses antibody appears at 6 months and with boosters leads to long-lasting immunity [23]. While the vaccine is effective, the time required to develop immunity is too long for defense against post exposure protection. It is recommended for adults who are at risk for exposure: laboratory workers, those exposed to infected animals, and some military personnel [25].

New vaccines under trial are directed to capsule antigens such as yDPGA® and conjugates with PA, subunits of PA, plant-derived PA and other approaches designed to enhance immunogenicity of PA, such as aluminum hydroxide absorbed PA. The vaccine blocks PA and inhibits toxin entering the cell (receptor blockade); antibody-mediated toxin neutralization directed to LR and EF is also an alternative.

#### 2.2.4. Polysaccharide vaccines

The next group of organisms has in common a polysaccharide capsule. Antibodies against the capsule can block infection. Immunization with the purified polysaccharide induces a weak and temporary T-independent antibody response. A strong long-lasting T-dependent response is induced using the polysaccharide antigen conjugated to a toxoid (diphtheria or tetanus). This induces long-lasting IgG antibody which blocks bacteria adhesion ...

### 2.2.4.1. Haemophilus influenzae

*Haemophilus influenzae* (Hib) is a Gram-negative bacterium that primarily colonizes the nasopharyngeal mucosa asymptomatically but can disseminate to other organs causing meningitis, pneumonia, epiglottitis and otitis media. Prior to use of vaccination Hib was a leading cause of childhood meningitis and pneumonia, causing up to 20,000 cases per year in the U.S. This has been reduced by 99%, eliminating Hib as a public health problem. The rate of invasive Hib disease decreased from 40 per 100,000 children to fewer than 1 per 100,000 [26]. Hib is covered with polysaccharide which allows it to be serotyped. Vaccines for Hib are directed to produce antibodies to the capsule. However, after immunization with pure PSP antibody levels fall rapidly after immunization [27]. The problem is that in this form the immune response is T-cell independent. Since antigen processing by macrophages and T-cell help are required for a long-lasting response, new vaccines were developed by conjugation of PSP to tetanus or diphtheria toxoid (Conjugate vaccines). This principle was then extended to meningococcus and pneumococcus, which have a similar pathogenesis. The immune mechanism is blocking of bacteria adhesion to mucous cells by polysaccharides. However, Hib exists in two major forms: typeable Hib that which expresses serologically typable polysaccharide (PSP) and non-typable HI (NTHi) which do not. NTHi adheres to and colonizes the mucous membranes in the nasal cavity through outer membrane proteins. Marked reduction in Hib infections following wide-spread vaccination was followed by an increase in H. influenzae infections by NTHi, which do not express PRP, presumably because of lack of competition for the infectious niche. To address this new threat, vaccines are being developed to other outer membrane proteins (adhensions, porins, etc.) that perform a similar function for NTHi that PSP accomplishes for Hib [28].

#### 2.2.4.2. Meningococcus

Meningitis and sepsis are caused by the bacterium *Neisseria meningitidis*; Phylum: *Proteobacteria*. It is a round organism that tends to form pairs (diplococcus). There are approximately 3,000 cases of bacterial meningitis per year in the U.S. and about 330,000 world wide. About 10% of adults carry the bacteria in the nasopharynx. The WHO recommend vaccination for high-risk countries and for travelers to these countries. Meningococcal vaccines have sharply reduced the incidence of the disease in developed countries [29]. The bacteria colonize and penetrate the nasopharyngeal mucosal membranes, pass into the bloodstream and are
transported to the brain where they multiply in the subarachnoid space of the meninges. This is followed by increased permeability of the blood–brain barrier, trans-endothelial migration of inflammatory cells and release of cytokines and prostaglandins [30]. Infected fluid from the meninges then passes into the spinal cord causing symptoms including stiff neck, fever and rashes. In some cases, there is bacterial sepsis with vital organ failure; even with antibiotic treatment about 10% of patients will die. There are currently three vaccines approved for use in the US. These are all quadrivalent targeting serogroups A, C, W-135 and Y. One was licensed in 1981 and is composed of purified capsular polysaccharide for each of the four subgroups. The other two were introduced in 2005 Memacta® (Sanofi Pasteur) and 2010 Menveo® (GlaxoSmithKline/Novartis) and are conjugated to diphtheria toxoid. These are recommended for large scale vaccinations when an outbreak of meningococcal disease occurs. Several new vaccines conjugated to tetanus toxoid are also under investigation and one of these Men AWY-TT (Nimenrix®/Pfizer) is recommend for travelers [31]. The vaccines are injected intramuscularly. Although still in use the polysaccharide vaccine is no longer recommended and is being phased out. Conjugation to a carrier protein changes the immune response from T-cell independent (mostly low-affinity IgM antibody) to T-cell dependent to produce a long-lasting high-affinity IgG response [32]. The objective is to produce complement-fixing antibody that causes lysis or opsonization of the bacteria (immune complex mechanism).

2.2.4.3. Pneumococcus. Pneumococcal infection is caused by a Gram-negative diplococcus: Streptococcus pneumonia. This organism lives naturally in the nasopharynx from where it can spread to the lungs, brain and systemically as well as be transmitted to others [33]. The mortality rates of pneumonia are highly variable (from 1 to 45%) [34]. Invasive pneumococcal disease kills thousands of people in the US each year (most over 65) and is the most common cause of invasive bacterial infection in children. The classic manifestation is lobar pneumonia with extensive consolidation of an entire lobe of the lung. Following the introduction of vaccines, the overall invasive pneumococcal disease decreased from 100 cases per 100,000 in 1998 to 9 cases per 100,000 in 2015 (CDC statistics). Of critical importance to the pathogenicity of S. pneumonia is its polysaccharide capsule which enables it to evade phagocytosis. Production of antibodies to this polysaccharide is the basis of the protection mediated by vaccination. The present vaccines target 13 or 23 serotypes of the bacteria (Pneumovax® (PCV) 13 or 23) and new vaccines are under development that will increase the coverage up to 72 serotypes [34,35]. The first vaccine was introduced in 1945 and included three serotypes using purified capsular polysaccharides. As with other polysaccharide vaccines this vaccine is T-cell independent, produces IgM antibody, short-term immunity and is ineffective in children. To change the immune response to T-dependent the polysaccharides are conjugated to diphtheria toxoid which induces IgG antibody, memory B cells [36], and life-long immunity. Immunization with PCV 13 protects against fewer serotypes but has greater immunogenicity that PCV 23 leading to the suggestion that both vaccines be used [37]. In the United States vaccination is recommended for adults 65 or over adults with health problems, smokers, and children older than two years with long term health problems [33]. PCVs have 76 to 92% efficacy. The protective immune mechanism is receptor blockade and antibody-mediated opsonization (Immune complex).

2.2.4.4. Streptococcus Group A (GAS). GAS are bacteria commonly found in throat and skin and usually cause a mild illness, such as strep throat and impetigo, but can occasionally cause necrotizing fasciitis (flesh eating bacteria), toxic shock, acute rheumatic fever or rheumatic carditis [38]. Approximately 20% of patients with necrotizing fasciitis and 60% with toxic shock die. About 10,000 cases of invasive group A streptococcal occur in the US each year resulting in about 2,000 deaths (NY.gov). Penicillin or erythromycin are effective treatments but do not cure each patient with invasive disease. Patients who recover from a GAS infection develop antibodies to GAS M-protein and have protective immunity. Clinical trials with heat killed organisms and crude M protein vaccines began in 1923 [39], with little success. Following the development of rheumatic fever in 2 recipients receiving crude M proteins in 1969, further trials were prohibited by the FDA for 30 years [40]. Clinical trials with multiple peptides (up to 30) from different M serotypes induce bactericidal antibodies and are well tolerated and safe [40–42]. Difficulties arise in assessing immunogenicity and tests such as phagocytosis are difficult and tedious [40]. More recently common M-antigens have been genetically engineered to provide limited epitopes that will induce opsonic antibodies (immune complex) [43], but not autoantibodies [40]. Epitopes contained in the hypervariable, type-specific N-terminus of the M-proteins appear to meet these criteria [40,44]. Variations in epitopes of M-protein have been extensively identified (emm types) and may be used to design future vaccines for specific geographic areas. An alternative approach is to construct vaccines to the conserved C-region of M-protein and induce IgA antibody (for example in the nose) that will inhibit binding to mucosa [45]. Additional vaccine candidates include pilin, fibronectin binding proteins [38], Protein F1/Sfb1, FbaA and other bacterial products that bind tissues [40]. The immune mechanism of choice for therapy is antibody-induced opsonization.

2.3. Receptor blockade and T-CTL

2.3.1. Viral exanthems

Viral exanthems are skin eruptions produced by reaction of T-cytotoxic cells with viral infected skin cells. Although starting as a systemic infection, lesions appear in the skin corresponding to the appearance of T-CTL. Lesions can vary greatly in severity with heavily inflamed progressive necrotic lesions (small pox) to mild self-limited flat erythematous patches (Rubella). Small pox is named because the lesions are smaller than the skin lesions of primary or secondary syphilis (large pox). Syphilis is not a viral exanthem.

2.3.2. Smallpox

Smallpox virus is in the family Poxvirus; genus: Orthopoxvirus; species: Variola virus. It has a single linear double stranded
DNA genome. Pox viruses are ‘proviruses’; they replicate in the cytoplasm of infected cells, not the nucleus. It has two forms enveloped and unenveloped; both are infective. The viral envelope is made up of modified Golgi membranes containing viral specific polypeptides. This complicates vaccine design, because antibodies to the envelope that might block cell entry would not act on viruses without an envelope [46]. Before vaccination, the risk of death following contracting the disease was about 30%, with higher rates among babies. Often those who survived had extensive scarring of their skin and some were left blind. In eighteenth-century Europe, it is estimated 400,000 people per year died from the disease, and one-third of the cases resulted in blindness. Smallpox is estimated to have killed up to 300 million people in the twentieth century and around 500 million people in the last 100 years of its existence. As recently as 1967, 15 million cases occurred in one year [47]. Vaccination against smallpox is one of the most important advances in human preventive medicine. The terms vaccine and vaccination originate from the use of Cow-Pox as an attenuated virus that induces Tcytotoxic cells that attack virus infected epithelial cells. Vaccinia virus is closely related to cow-pox virus and were once considered to be the same. The Latin name for Cow Pox is Variola vaccinae: the term vaccination was extended to include all forms of protective immunization. Vaccination against smallpox is accomplished by introducing the attenuated virus into the epidermis with multiple needle punctures. If the vaccination is successful there is a crusting superficial contact dermatitis (e.g. Poison ivy) like inflammation about a week after inoculation. This is termed a ‘take’ reaction and indicated successful induction of immunity to smallpox. This process is so successful that smallpox as a disease has been essentially eliminated and vaccination discontinued because of rare morbidity and mortality. However, stocks of the virulent virus remain in laboratories in the U.S. and Russia. The possibility of weaponizing smallpox has led to an increased interest in vaccine improvement [46]. In 2002, the United States military resumed smallpox vaccinations as a precaution to weaponized use. Since then at least 4 cases of life-threatening infections have occurred in individuals with eczema who have had contact with vaccinated individuals [47]. Historically smallpox probably was responsible for more deaths at early ages than any other disease. In epidemics up to 50% of the children in a village might die. In ancient China names were not given to infants until after they survived smallpox because so many children died that families would run out of cherished family names [48]. The Chinese noted that milder forms of the disease resulted when individuals were infected with virus from others with a relatively benign course of the disease.

About 1720, Lady Mary Wortley Montague, the wife of the British ambassador to Turkey, observed this process and returned to England convinced of its efficacy [49]. She had her 4-year-old daughter intentionally infected with virus from a mild case of the disease and she was subsequently protected against smallpox. She persuaded King George I to conduct trials on prisoners at Newgate Prison. This proved highly effective in preventing smallpox reducing mortality rates to as low as 1%. The process became known as variolation or inoculation and soon was widely practiced in Great Britain. During the revolutionary war the American army was decimated by smallpox, but the disease was controlled by variolation. However, because virulent virus was used, the disease became endemic and would never be eliminated [49]. Variolation involves direct infection of the epidermis of the skin so that an immune response is initiated before systematic infection occurs [49]. Once inhaled, variola major virus invades the oropharyngeal (mouth and throat) or the respiratory mucosa, migrates to regional lymph nodes, multiples and moves from cell to cell. By the 12th day or so, lysis of many infected cells occurs and the virus is present the bloodstream in large numbers (viremia). A second wave of multiplication occurs in the spleen, bone marrow, and lymph nodes. The infection then spreads to the mouth and skin with formation of a skin rash that progresses to fluid filled bumps that rupture and scar. By introducing the infection in the epidermis, the immune response gets a head start on the systemic infection. In 1796, Edward Jenner, a simple country physician from Gloucestershire, observed that Sarah Nelmes, a dairymaid developed a sore on her hand from Cow Pox infection became resistant to smallpox [49]. Further observations on similar Cow Pox infections convinced Jenner that relatively benign limited infection with Cow Pox was superior to variolation as fatalities were essentially eliminated and use of the attenuated virus removed the necessity to employ the virulent smallpox virus (variolation). Even though there was considerable resistance to this [50], like the present reaction to measles and influenza vaccines today, The WHO launched universal vaccination as the means to eliminate smallpox. The last known case was reported on Somalia in 1977; the WHO certified the global eradication of the disease in 1980 [47].

2.3.3. Measles

Measles is a highly contagious systemic infection with the main manifestation of multiple inflammatory lesions in the skin (rash). Wild-type virus is spread though inhalation of respiratory aerosols. Infected macrophages spread the infection to multiple organs (skin, kidney, lung and liver, etc.). The skin lesions are a manifestation of T-cytotoxic cells invading and killing infected epidermal cells. (T-cell cytotoxicity). Before vaccination measles was responsible for more than 2 million deaths annually; now about 100,000 globally. Although declared eradicated in the U. S. in 2002 [51], resistance of some families to vaccination reduced herd immunity and by June 2019 over 1,000 cases were reported in the US. The current vaccine (MeV) is an attenuation of wild-type virus by in vitro passage in human and chicken cells and is effective in inducing both antibody and T-cytotoxic cells [52]. It is usually administered as a quadrivalent vaccine for measles, mumps, rubella and varicella. The vaccine is effective in inducing both protective antibody to the H protein as well as CD8+ T-CTLs. The antibody to H is required for blocking the reaction of the WT virus with cell surface receptors (SLAM). Both antibody and T-cytotoxic cells are necessary for effective immunity. The vaccines are safe and effective and universal application could lead to eradication of the disease.
2.3.4. Rubella (German measles)
Rubella is a viral exanthem caused by the only member of the togavirus family. The virus is encapsulated and has a single stranded RNA genome [53]. Most adult cases are mild with a generalized rash that occurs about 2 weeks after exposure, lasts about 3 days and is less red than measles. It was first described in Germany and has become known as German measles or three-day measles. Infection during early pregnancy may lead to still birth or the congenital rubella syndrome (CRS, cataracts, deafness, heart and brain defects). The virus infects the placenta, spreads to the fetus and interferes with organ formation [54]. The nature of the receptor on infectious cells is not known, but it appears to be able to bind and infect various epithelial cell types. Rubella (from Latin ‘little red’) is spread through the air through inhalation, rapidly disseminates and can infect many other organs. In adults this is prevented by the immune response, but in fetuses there is dissemination to many organs [53]. Only humans are infected; there are no animal or bird carriers. The rubella vaccine in a live-attenuated virus produced by passage in tissue culture. A single dose is more than 95% protective [54]. Vaccination is highly recommended for women of child bearing age. However, it is now part of the standard childhood vaccination program and with other viruses (MMR). The incidence of rubella has dropped sharply following introduction of vaccination. During an epidemic in the early 1960’s in the US rubella infections caused 30,000 stillbirths and 20,000 children with CRS [55]. Since vaccination was introduced the number of cases in the Americas dropped to 3,000 a year and the last reported case was reported in 2009. However, there are still about 100,000 cases of CRS per year in the rest of the world [54]. A vaccination rate of 80% should establish herd immunity and prevent any further infections. Later after immunization or when the exanthem appears adaptive active cell-mediated immunity is seen (increased TNF-α and IL-10) [56]. The exanthem indicates T-cell cytotoxicity of infected epithelial cells, an effect that is most likely taking place on other infected cells in the body. Protective immunity is mediated by antibody neutralization of receptors to block infection of cells and therapeutic immunity by T-cell cytotoxicity of infected cells.

2.3.5. Varicella
Varicella zoster virus (VZV) is a member of the alpha herpesvirus family of DNA viruses. It has linear 125-kb double-stranded DNA genome encapsulated by a polyamine, lipid and glycoprotein envelope that binds to receptors on cells and allows entry [57]. It causes two distinct diseases: chicken pox and herpes zoster (shingles). The virus initiates infection in the oropharynx and is transmitted systemically to skin epithelium via infected T cells [58]. Appearance of the typical skin rash correlates with specific T-cytotoxic cells that target the surface glycoprotein and various viral proteins. These cells are major mediators of epidermal injury and protect against future infection [58]. Decreased immunity with aging allows recurrence of infection in sensory nerves (herpes zoster). VZV is ubiquitous and before a vaccine was available essentially everyone developed chicken pox at some time in their life. In most instances this was a relatively mild self-limiting infection; but it could become life threatening in patients with T-cell immune deficiencies or rarely in normal individuals. The first live-attenuated vaccine, vOka* was developed by serial passage of the wild-type virus in human and guinea pig embryo-fibroblast and human diploid cells in Japan in 1974 [59]. In further trials it proved to be safe and efficient [60]. Current vaccines (Vairavax®.OKA/Merk) and Varilrix® (OKA/GSK) are derived from vOka after passing in cell culture and combined with measles-mumps-rubella-varicella live-attenuated vaccines [61]. This combined vaccine is >90% effective and has very rare side effects [61,62]. The major protective immune response is IgA antibody inhibition of infection in the oropharynx; IgG antibody can prevent spread of the infection by inhibiting binding to target cells and T-CTL by killing infected cells.

2.3.6. Influenza
Influenza is included in the category of viral exanthems because the virus infects bronchial epithelial cells and type II pneumocytes which are then attached by T-cytotoxic cells leading to what could be called ‘viral exanthem of the lungs’ [63,64]. Influenza is caused by a highly mutable virus that is spread through inhalation of contaminated air emitted from infected patients. It produces about 1 billion infections annually of which 35 million are severe, and up to 500,000 deaths. Vaccination is the primary approach to control and prevention. Because of transcription errors point mutations result in different variations of surface antigens each year with selection of the new variants as the susceptible population develops antibodies to the previous variants [65]. Vaccines are either killed/inactivated or live-attenuated influenza viruses. Due to emergence of different antigenic variants new influenza vaccines are introduced each year to cover the new variants. The vaccines induce neutralizing antibodies to the globular head of viral hemagglutinating and neuraminidase antigens. In the past, the vaccine contained 3 antigens (trivalent); quadrivalent vaccines are now being introduced to provide a wider range of protection. The live-attenuated vaccines are administered intranasally and induce both local secretory IgA and circulating IgG antibodies, as well as T-cell responses. However, the effectiveness of the live-attenuated vaccine has been questioned over the last few years and killed/inactivated viruses were recommended. A live-attenuated vaccine (FLUMIST®) was approved for the 2018–2019 flu season [66]. Recently experimental models have defined the role of T-cell-mediated cytoxicity as a means of killing infected bronchial mucosa and type-II pneumocytes analogous to the killing of infected epidermal cells of the skin in viral exanthems [63,64]. An alternative to targeting surface molecules that are highly mutable is to substitute internal structural proteins, which remain constant and induce both CD8 and CD4 T-cell responses. The induction of cytoxicity may be enhanced by using recombinant vaccinia virus as vector [64].

2.3.7. Mumps
Mumps is cause by a paramyxovirus transmitted by inhalation. After a 12 to 15 days incubation period there is painful swelling of the parotid gland, the classic finding. (parotitis) that may involve other organs such as meningitis, orchitis, oophoritis, mastitis and pancreatitis. Historically mumps is a childhood infection [67,68]. Parotitis may not always be present and diagnosis
depends on laboratory tests (RT-PCR for virus or IgM seroconversion). Mumps epidemics occur about every 4–5 years mainly among children 5–7 years of age with about 6,000 cases per epidemic in the US [69]. The disease is usually relative mild but aseptic meningitis and orchitis can be serious complications. The disease is largely contained by vaccination. In the US the number of cases reported per year has declined from over 150,000 to about 300. The mumps vaccine is a live-attenuated virus originally from the Jeryl Lynn strains of mumps virus cultured in embryonic hens’ eggs and chick embryo cell cultures by Maurice Hilleman obtained from his daughter Jeryl Lynn when she had mumps in 1963 [70]. Two doses of the vaccine are 88% effective in preventing disease, so in epidemics many patients may have been vaccinated [71]. Other attenuated strains are available, particularly in Russia, but are less effective and have more side effects. The vaccine is believed to induce neutralizing antibodies that block binding of the surface glycoprotein-hemagglutinin-neuraminidase and fusion protein to glandular target cells. T-cytotoxic lymphocytes to virus infected ductal cells may also be necessary for complete protection [71].

2.3.8. Ebola

Ebola virus disease is a viral hemorrhagic fever. There are four viruses in the genus 
Ebolavirus that infect humans, named for the geographic areas first identified: Bundibugyo, Sudan, Tai Forest, and Zaire [72]. The Zaire virus, now commonly called just Ebola, is responsible for most human infections. The virus spreads through direct contact with contaminated fluids, such as blood, saliva, vomit, sweat, etc. It replicates in many cells, including endothelial cells, monocytes, liver cells, fibroblasts, etc. and causes apoptosis of immune cells and a weakened immune response. The virion begins infection by using a viral glycoprotein GP to attach to specific cell-surface receptors such as Ctype lectin DC-SIAN followed by fusion of the viral envelope with cellular membranes. The virions are taken up by the cell and then processed and the RNA transcribed into positive strand RNAs. After infection, there is synthesis of Ebola virus glycoprotein which reduces specific intergrins and causes loss of vascular cell adhesion and hemorrhage. Left untreated over half of those infected will die. One of the first outbreaks occurred in a village near the Ebola river in the Democratic Republic of the Congo. Since 1976 there have been regular outbreaks including the major one from December 2013 to March 2016 with 28,616 cases and 11,310 deaths [72]. Organizations, including the WHO, NIH, The Gates Foundation and others funded a ‘space race’ program to develop a vaccine for Ebola. In a relatively short period of time vaccines have been constructed and tested [73]. The first was inactivated whole virus but this never went to trials because of safety and efficacy deficiencies. More sophisticated approaches utilize the envelope glycoprotein (GP) of EBV. The two leading candidates that entered clinical trials are chimpanzee adenovirus 3 (ChAD3) and vesicular stomatitis virus (VSV) vectors encoding the GP from the Ebola virus. The VSV vaccine (rVSV-ZEBOV®) has proved to be both safe effective in ring vaccination clinical trials where both infected individuals and others in the community are immunized. This vaccine is currently being used to contain an outbreak in the Congo. It has proved to be 97% effective and almost certainly has prevented a global threat [74]. Even so there have been 1,206 cases and 764 deaths since 2018, but this is far less that the 2016 outbreak and logistical and safety problems. Laboratory studies support the protective effect due to antibody-mediated blocking of the binding of viral GP to host cell surface receptors [75]. Since virus vectors are used for immunization, it is likely that there is also T-cell cytotoxicity.

2.3.9. Rotavirus
Rotavirus infects every child in the world and is the leading cause of severe diarrhea among young children, accounting for 20% to 30% of severe diarrheal disease that requires hospitalization. There are more than 114 million rotavirus diarrheal episodes, 24 million clinic visits, 2.4 million hospitalizations and 500,000 deaths per year in children younger than 5 years of age [76]. Rotaviruses are large particles with three concentric protein layers that surround the viral genome [77]. The virus contains six structural proteins and six proteins that are synthesized in the infected cell and function in viral replication or interact with host protein to influence pathogenesis and the immune response. Viral proteins 7 (VP7) and VP4 bind to small intestine enterocytes, contribute to serotypes and are targets for blocking antibodies [77]. There are at least 11 VP4 types and 10 VP 7 serotypes, but 90% of infectious viruses belong to just four combinations. Rotavirus vaccines first became available in the US in 2006 and prevent 15–35% of severe diarrhea in the developing world and up to 96% in the developed world. The vaccine should be used in high-risk areas and among food workers [78]. There are 6 rotavirus vaccines that have been studied [69]. The most widely used is RotaTeg®, which is a live, oral attenuated pentavalent vaccine produced by recombination (FDA approved in 2006). An improved attenuated virus produced by multiple passages in culture (Rotarix®) produces long-lasting immunity after fewer doses and is safe and effective in clinical trials: 85% effective against severe diarrhea and 100% effective against the most severe cases [79]. Naturally acquired immunity correlates with intestinal IgA antibody and IgA memory cells. The vaccines are administered orally to facilitate the local IgA response. The major immune protective reaction is antibody neutralization of virus-target cell binding, but other mechanisms may contribute including natural immunity and T-cell cytotoxicity.

2.3.10. HIV AIDS

Human immunodeficiency virus (HIV) is a member of the genus Lentivirus and family Retroviridae. HIV AIDS is transmitted by sexual contact or between a mother and fetus. It continues to be an increasing world-wide plague. Although retroviral therapy has resulted in a decrease of infections from 3.3 million in 2002 to 2.3 million in 2012, there is a continued increase in the number infected as therapy has resulted in many people living longer with subclinical HIV infections [80]. Like Herpes, HIV infection is for life; it is not cured, only held in check by retroviral therapy. Immunity to HIV is complicated by the fact that the target cells: CD4 T cells, macrophages and dendritic cells, are the same cells that take part in the immune response to HIV. Most effective vaccines are whole killed organisms or attenuated organisms; killed HIV is not
sufficiently antigenic and there are safety issues with a live retrovirus vaccine. Two vaccine approaches are proposed: antiENV neutralization and T-cell cytotoxicity [81,82]. The development of a neutralizing vaccine has proved to be very complicated because of the genetic diversity receptor for infectible cells and the nature of the HIV life cycle. Early trials on inducing antibodies to selected HIV ENV proteins were ineffective [83]. The next generation of clinical trials utilized the AIDSVAX B/E envelope protein with a canary pox virus carrier (RV144). This yielded an efficiency of 31.2%; too low for licensing [83]. The diverse envelope (ENV) states involved in HIV-1 entry into HIV target cells pose a problem for vaccine specificity. The proteins include a fusion envelope protein closed state, a single CD4-bound intermediate, a three-CD4-bound intermediate, a pre-hairpin intermediate and post-fusion states. These provide multiple epitopes for antibody specificity [82]. It is not yet clear which epitopes are critical for receptor blocking antibodies and it is unlikely that a single antibody specificity would be effective. This understanding came from study of hundreds of humoral antibodies that were found in patients after five or more years of infection, termed broadly neutralizing antibodies (bNAbS). The conformation of the ENV for optimal vaccine design for antibody neutralization is under investigation [83]. An additional approach is DNA in viral vectors (recombinant cytomegalovirus, adenovirus and vaccinia virus) to induce CD8 + T-cytotoxicity directed to infected cervical-vaginal mucosa [81]. It is argued that this local immunity could be critical to preventing spread of HIV infection. Development of an effective vaccine to HIV has a long way to go, but the resources and expertise being applied should be productive of an effective vaccine in the foreseeable future. It is likely that an effective vaccine will need to include receptor blockade and CD8 + T-cell cytotoxicity.

2.3.11. Rabies

Rabies is an infection of the central nervous system by viruses of the *Lyssavirus genus*. Human infection usually follows a transdermal bite or scratch by an infected animal.

Rabies in dogs is responsible of 99% of human infections and poses a potential threat to 3.3 billion people. The inoculated virus enters peripheral nerves and sometimes muscle cells at the motor endplate of the neuromuscular junctions by endocytosis and is then transported to the central nervous system through motor neurons [84]. In the brain the virus replicates and then, after 1–3 months, it is distributed systemically where it thrives in the salivary glands [85] and is available for transmission by biting. In the CNS, the infection results in progressive fatal encephalomyelitis characterized by hyperactivity, fluctuating consciousness, and in severe cases, hydrophobia and death by cardiac arrest.

Vaccination against rabies is applied both therapeutically and preventatively. Because of the relative long latent period after infection, the vaccine is used to induce an immune response in infected individuals before the virus can propagate in the brain and cause symptoms. In some cases, if it appears that it will take too long for the primary immune response to occur, immune globulin is recommended along with immunization. Vaccination is also recommended for individuals at risk, such as veterinarians or farm workers. One of the most important applications is the universal immunization of dogs. This has been very effective in prevention of rabies by ‘herd immunity’. In the United States, rabies has been essentially eliminated by canine vaccination [85]. Vaccines for rabies consisting of dried nerve tissue derived from infected rabbits were introduced by Louis Pasteur and Emile Roux in 1885 with initial success in treating a nine-year-old boy bitten by a dog [86]. The virus produces 5 surface proteins, the most critical for an immune response is the G protein which functions for endocytosis and is highly antigenic [85]. The present vaccines are derived from inactivated, lyophilized virus cultured in human cells. The use of these vaccines has eliminated the necessity for multiple injections required by earlier vaccines which has markedly improved patient participation [84]. All licensed vaccines have good safety records and are highly effective. The immune effector mechanism is antibody-mediated neutralization (receptor blockade) of the virus including local antibody production. Production of rabies virus antibodies by B cells infiltrating across the blood–brain barrier is crucial for elimination of the rabies virus from the brain [87]. In addition, T-TCL elimination of infected cells may be critical but may also contribute to loss of neurons.

2.3.12. Hepatitis

2.3.12.1. Hepatitis A. Hepatitis A virus is picornavirus that is passed by fecal-oral route in contaminated food and water. Hepatitis A rarely causes chronic liver injury as does Hepatitis B, but with few exceptions, produces an acute self-limiting illness. Like all hepatitis viruses, the virus has affinity for the liver, i.e. it is hepatotropic. Although during infection the virus can be found in other organs, pathologic changes are limited to the liver and are the result of the host immune response to the infected liver cells [88]. There are both apoptosis and periportal piecemeal necrosis characteristic of autoimmune hepatitis mediated by T-cytotoxic cells. Piecemeal necrosis extends from the periportal region gradually across the liver cord and consists of lymphocytes surrounding and killing individual hepatocytes. Plasma cells may also be seen in areas of inflammation and antibody produced to the virus results in long-term immunity after infection by receptor blockade. There is a very high incidence of hepatitis A in endemic areas with most children asymptomatically infected before adulthood. Of these, about 1% develop liver failure. The introduction of vaccines in the 1990's has had a major beneficial effect. The estimated number of cases has declined by 94–97% in the United States, but there still may be over 1.5 million cases per year worldwide [89]. Vaccination works by induction of circulating antibody that prevents infection of hepatocytes; again, these antibodies do not act on already infected cells. This requires T-cell cytotoxicity as is the case for other hepatitis virus infections.

2.3.12.2. Hepatitis B. This hepatotropic virus has a high incidence in endemic countries with poor sanitation (almost 120 million in the western Pacific region and 60 million in Africa), with a much lower incidence in North and South America (less than 5 million) [90]. During pregnancy it is
frequently transmitted from infected mothers to their fetuses. In these children and those infected before 2 years of age, with an immature immune system, infection produces a chronic condition with liver failure and a high incidence of liver cancer. Complex immune mechanisms lead to chronic persistent infection [82], but a common feature is an attenuated T-cell response. In adults, an acute febrile illness, usually resolving but with rare liver failure is more common. HBV has both low- and high-affinity binding to hepatocytes which can be blocked by specific antibodies to HBV. The virus is not cytopathic; liver damage is mediated by T-CTLs reacting with infected hepatocytes and features piecemeal necrosis. Four to 7 weeks after infection hepatitis B surface antigen (Australian antigen [91]) may be detected in the serum whereas later in infection this is replaced by antibody to HBsAg. Circulating antibody has a role in suppressing chronic infection but does not affect already infected cells. Patients treated with anti-B cell therapy (rituximab®) have a high risk of viral reactivation. Vaccination has had a marked impact on HBV infection. Because of the risk of neonatal infection, the WHO recommends not only vaccination of mothers, but also newborns within 24 hours after birth. There are 2 effective and safe vaccines. The first was HBsAg isolated from serum of asymptomatic carriers [92]. The second was from cloned HBV DNA fragments encoding the S protein [93]. It may be administered separately or in combination with DPT and polio [89]. There are varied doses and schedules recommended but the vaccine is effective in infants, children and adults with high risk [94].

Even so, HBV remains a major public health problem worldwide and is the major contributor to liver cirrhosis and liver cancer. In contrast, with application of an aggressive policy of universal vaccination for HBV there has been a dramatic decline of acute HBV in the United States [89].

### 2.3.12.3. Hepatitis C

HCV is a major cause of morbidity and mortality worldwide accounting for over 54,000 deaths and almost 1 million disability adjusted life-years [89]. Therapy and prevention are complicated by the fact that there are seven known genotypes [95] and there is rapid error prone replication in vivo resulting in production of numerous replicants that can avoid the host immune response and response to therapy [95]. There is no effective vaccine and treatment depends on interferon and new direct acting anti-viral drugs [89,95]. Both CD4+ and CD8 + T cells are required for viral clearance, and the role of antibody is not clear [96]. There is a detectable antibody response within 1 month of infection, but the intracellular virus is not affected by humoral antibody. On the other hand, remission is associated with prominent CD4+ and CD8 + T-cell responses. Modified vaccinia and adenoviral vectored vaccine encoding for proteins of HCV has been used to generate HCV specific T cells and clinical trials are now underway [97].

### 2.3.12.4. Hepatitis E

Hepatitis E virus has four genotypes, with types 1 and 2 most likely to infect humans. The WHO estimates that there are 29 million infections worldwide with 44,000 deaths, mostly in resource poor areas including China, North Africa and Central America. The virus is shed in the stool resulting in fecal-oral spread through water contamination. The disease usually resolves in 2–6 weeks but can cause up to 30% mortality in pregnant women [98]. The immune response is humoral neutralizing antibody directed against an open reading frame (ORF-2) that encodes the antigenic capsid protein. A vaccine (Hecolin®) based on this antigen has proven 88% effective in China [98] but has not been commercialized because it is thought that it will not be profitable [99]. The WHO has determined that further safety and efficacy studies should be done before the vaccine is licensed [99].

### 2.3.13. Polio

Polioymelitis is caused by an enterovirus that enters the nasopharynx and GI tract where it infects macrophages and overlying epithelial cells in tonsils and Peyer’s patches that express PV receptors (CD155) [100,101]. The virus replicates in these cells and are shed into the GI tract or carried to the central nervous system. From descriptions of the histopathology in experimentally infected non-human primates [102], one can speculate that T-cell-mediated activation of macrophages (delayed hypersensitivity) eventually arms the infected macrophages and T-cell-mediated cytotoxicity is directed to the infected enterocytes to cure the gastrointestinal and systemic infection, but usually not before the virus has spread to the CNS. Circulating antibody induced by polio vaccination cannot act on the infected monocytes or epithelial cells but could block infection of monocytes, and prevent the virus from spreading to the anterior horn cells of the spinal cord. There, TCTL to infected cells causes death and failure of these cells to send signals to skeletal muscle and flaccid paralysis. Prominent is lymphocytic infiltration with eventual phagocytosis and destruction of the infected cells [103]. In many other infectious diseases T-cell responses are key to clearing infected cells and recovery (for example, small pox, measles, influenza, syphilis, tuberculosis, etc.). In other infections, there is redundancy or the involved tissue cells have the capacity for rapid replacement. However, there is no replacement capacity or redundancy in the large motor neurons of the CNS.

Paralytic polio is a disease of the twentieth century. Prior to widespread sanitation most newborn infants became infected with polio before appearance or myelination of the anterior horn cells. In this instance antibody production occurred before the anterior horn cells developed sufficiently to be infected. Before sanitation polio was relatively rare and did not appear in epidemic form, usually occurring sporadically in 2–4 years old children. On the other hand, in the twentieth century, many children and young adults were not exposed to the virus until after the anterior horn cell were sufficiently mature to become infected. In the early twentieth-century widespread epidemics became frequent involving older children and young adults. By the twenty-first century, world-wide application of polio vaccines essentially eliminated the disease except for pockets in remote places usually afflicted by strife or religious objections to immunization [104]. Two vaccines are available for polio, formalin killed (Salk) and attenuated live virus (Sabin). Both produce effective immunity, but each has an advantage and disadvantage [104]. The attenuated virus in the Sabin vaccine infects gastrointestinal cells and produces local immunity as well as systemic immunity. The Salk vaccine does not induce local immunity so that even if the individual is protected from neuronal loss, the wild-type
virus can reproduce in the gastrointestinal tract, proliferate and spread to others; herd immunity is not established with the Salk vaccine. On the other hand, there is a low but significant morbidity and mortality with use of the Sabin vaccine, but not with the Salk vaccine.

The effectiveness of the vaccines is unquestionable. The incidence of the disease in the United States dropped from over 22,000 cases per year to 4,594 after introduction of the Salk vaccine to 468 when both vaccines were used and from 1973 to 1978 and with widespread use of the Sabin vaccine to nine cases per year [105]. There are now approximately 400 cases per year worldwide, but also a few isolated incidences were reinfec-
tion is imported [104]. Because of rare infections events following oral poliovirus vaccine (OPV), most countries have shifted to inactivated polio virus vaccine (IPV). With the overwhelming success of polio vaccination worldwide more cases are caused by OPV than by wild-type virus. The WHO, the United Nations Children’s Fund and Rotary International developed a Polio Eradication and Endgame Strategic Plan to wipe out polio by the year 2018 (the original goal was by 2000). According to the WHO as of March 2019, 33 cases were identified in 2018. Therefore, it is so close, yet so far away. It is likely that the IPV will continue to be given for some time until there are no more cases of WT infection. At this time, there is continued fine tuning of vaccine type, dose and schedule to determine the best way to maintain complete eradication [104].

2.3.14. Chagas disease
Chagas disease (American trypanosomiasis) is an inflammatory cardiopathy caused by Trypanosoma cruzi transmitted by kissing bugs (reduvid bug). In addition to humans, natural hosts include domestic and wild animals. In Latin America, there are an estimated 9–10 million infections per year with over 10,000 deaths and 100,000 years lived with disability [106]. About 70% of infections are asymptomatic, in the remaining there may be an asymptomatic stage lasting 4–8 weeks, but in some there is a localized swelling at the infection site (Chagoma). Disease manifestations include arrhythmia, aneurysms and heart failure and/or gastrointestinal dilation and hypertrophy. Death is usually due to ventricular tachycardia or fibrillation [107]. Trypanosoma cruzi enters the host through deposit of contaminated feces into fresh bite sites or mucosal surfaces as trypomastigote forms, and infects epithelial cells, fibroblasts and macrophages. After initial rounds of replication near the site of inoculation the released trypomastigotes infect a wide range of cell types throughout the body [107]. The parasites develop into the epimastigote form in the midgut. These multiply and are released back into the colon as trypomastigotes where they are passed into the feces. During infection the parasites show affinity to the heart and can multiply massively there. This causes damage to myocardial fibers and intense cellular inflammatory infiltrate changing from acute to chronic featuring T cells and macrophages and non-necrotizing granulomata with giant cells. Affected hearts become markedly enlarged with aneurysm formation. The progression of cardiac lesions continues even as the number of infecting organisms decreases, but some patients develop cardiac autoimmunity [107]. In addition to natural immune mechanisms, Trypanosoma cruzi infections feature Th1-type CD4+ helper cells with prominent development of CD8 + T-cytotoxicity responses that clear infected cells [108,109]. Chemotherapy for Chagas disease, benz-nidiazole or nifurtimiz is highly effective if given early after infection, but becomes increasing ineffective as the infection progresses. There are two approaches to vaccines; preventive and therapeutic [110]. Preventative vaccines could be directed to blocking binding of trypomastigotes to cell surface receptors. Therapeutic vaccines would need to be directed to CD8 + T-CTLs inducing a cytotoxic cell response to remove infected cells combined with chemotherapy [110]. Present pre-clinical vaccine candidates include recombinant proteins, attenuated organisms, adenoviral and salmonella vectors. Since T-CTL responses might be best for both preventative and therapeutic vaccines, cutaneous administration of a vaccinia-vectored vaccine should be considered.

2.3.15. Epstein Barr virus
Epstein-Barr virus is the causative agent of infectious mononucleo-
is an inflammatory disease caused by EBV-infected transplanted organs. Potential T-cell immunogens include EBV glycoproteins, lytic proteins, and latent proteins. The primary candidate has been EBV glycoprotein gp350 through which EBV binds to CD-21 on B cells [112]; antibody to gp350, which acts to inhibit infection (neutralization mechanism) or as a target for cytolytic T cells. T-cytotoxicity may limit the first stage of infection of the epithelial level and may block reactivation of proliferation in B cells [113]. This is especially important in immunosuppressed transplant patients where infection arises from the EBV-infected transplanted organ. Potential T-cell immunogens include EBV early response proteins Zta and Rta [114], lytic proteins, structural proteins [115], or EBV latent proteins expressed on infected B cells, such as EBV nuclear antigen (EBNA1) and leader protein (EBNA-LP). A Phase-II trial of gp350 in alumn/MBL reduced the rate of infectious mononucleosis by 78% [115]. Two new vaccines have been developed; one to elicit antibodies to gH/gL, a protein on epithelial cells that fuses with EBV [116], and another against a viral protein (gp42) that enhances fusion of the virus with B cells [117]. These approaches have not yet been tested in clinical trials.

2.4. Receptor blockade and DTH
These are grouped together because there is a common protective strategy: preventive, antibody-mediated receptor blockade (neutralization) and therapeutic (CD4 + T-cell-mediated delayed hypersensitivity, DTH). However, differences in the pathogenesis and immune response reflect variations in the natural history of the infection
2.4.1. Yellow fever

Yellow fever is caused by a member of the RNA Flaviviridae family which includes yellow fever, Japanese encephalitis, West Nile and dengue viruses. These are transmitted by mosquitoes in endemic areas. Upon inoculation into the skin the virus disseminates to the draining lymph nodes where following clathrin-dependent endocytosis into mononuclear cells, particularly monocytes, the genome is rapidly translated, processed and productive of infective virus [118]. Then the virus reaches the liver and infects hepatocytes, most likely mediated by infected Kupffer cells, and causes apoptosis of the liver, accentuated by NK cells [119]. After a short incubation, symptoms include fever, muscle pain and headaches, which usually improve within 5 days. However, after this resolution symptom recurs more severely in about 15% of infected individuals and liver damage leads to yellow skin (jaundice) and about half of those with recurring illness will die [120]. Historically, yellow fever became prominent in the nineteenth century [121]. It was introduced into South America through the slave trade in the 1800’s and the higher incidence in the disease in slaves when compared to Europeans led Napoleon to sell the Louisiana Territory to the United States in 1803, with a massive epidemic occurring in Memphis, Tennessee in 1878. Soon after this Walter Reed demonstrated that the disease was carried by mosquitoes. Control of the disease by elimination of mosquito transmission greatly facilitated the construction of the Panama Canal [121]. The first vaccine to yellow fever was developed in 1927 from attenuation of wild-type virus by passage in mouse brain B cells. In the 1940s, such a strain, called the French strain, was developed at the Institute Pasteur and used extensively in French West Africa, but it’s association with post-vaccinal encephalitis led to discontinuation in 1981. In the meantime, another strain, the Asibi strain, named after the patient from which it was isolated in Ghana, was used at the Rockefeller Institute to produce an attenuated stain after 17D, may stimulate CD4 + T cells to activate infected macrophages [118]. A rare but often severe and fatal reaction to 17D vaccination is yellow fever-associated viscerotrophic reaction (YE-AVD) and efforts are underway to identify those at high risk for this reaction to improve safety [121].

2.4.2. Japanese encephalitis

Japanese encephalitis is caused by a single-stranded RNA virus that is transmitted by the Culex mosquito. The virus enters the central nervous system via infected lymphocytes or monocytes where the virions bind to the endothelial surface, are internalized by endocytosis and passed to neurons [122]. Humans are dead-end hosts for the virus; infected humans cannot transmit the virus to another mosquito. The natural animal hosts are birds and mammals, especially pigs. Although very common in endemic areas only about 1% of infected individuals develop clinical disease [123]. There are 30,000 to 50,000 cases per year with a death rate of 30% [124]. Culex mosquitoes live throughout South-East Asia and in the tropics, Middle East and Africa. Because there is no effective anti-viral treatment and prevention by elimination of the carrier mosquitoes is difficult, vaccination is considered as the most effective way of preventing the disease. Various vaccines, including inactivated mouse brain derived virus, live-attenuated viruses, and formalin-inactivated virus, which had acceptable, but not safe or effective have been tested. The present IC51 vaccine is a purified, inactivated whole virus (IXIARO®). It was derived from an attenuated virus (SAL14-14-2), cultivated in Vero cells and formulated with 0.1% aluminum hydroxide. This has proven to be safe and 90% effective [124]. At present, the vaccine is underutilized and there are recommendations to expand use in endemic areas and travelers to endemic areas [125]. The vaccine induces high titers of neutralizing antibody that prevents infection of susceptible cultured cells. A key antigen is NS1, a glycoprotein secreted during viral replication. Anti-sera to NS1 prevents experimental infection in mice [126], but CD8 + T-cell cytotoxicity and CD4+ activation of macrophages (INF-γ) may also be active in recovery from infection [127]. In any case, the major effect of vaccination is to prevent infection by blocking virus binding to host cells.

2.4.3. Dengue fever

Dengue fever includes a high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash. It begins about 2 weeks after being bitten by an infected mosquito [128]. About 80% of exposed individuals have no symptoms. Upon injection the virus binds to and infects Langerhans cells in the skin which migrate to draining lymph nodes. The infection is passed on to circulating monocytes where the virus multiplies [128]. Many of the symptoms of Dengue fever are believed to be due to cytokines and interferons released from these infected cells. Unusually severe forms include dengue hemorrhagic fever and dengue shock syndrome. The Dengue virus has 4 serotypes for which a vaccine must be directed. Several vaccines are under investigation, including live attenuated, inactivated, DNA and subunit vaccines. Live-attenuated vaccines are the furthest along [129]. In 2016 Dengvaxia®, a live-attenuated tetravalent vaccine made by recombinant DNA processing was used in high-risk countries with partial success. A major problem is that seronegative recipients have a high risk of severe dengue after immunization, so it is only recommended for seropositive individuals, essentially acting as a booster immunization for those who have recovered from Dengue. Ongoing trials in Latin America and Asia reveal an efficacy of about 60% in seronegative recipients and over 80% in seropositive. Several other vaccines are under development [130]. Since the virus replicates in monocytes and not epithelial cells immunization that favors DTH (BCG vector) might be
effective in activating the infected monocytes to eliminate the infective viruses.

2.4.4. Chikungunya virus
Chikungunya virus (CHIKV) is a positive sense RNA togavirus transmitted by *Aedes* mosquitoes. It causes an acute febrile illness followed by debilitating polyarthralgia of the peripheral joints like Dengue virus [131]. Prior to 2004, CHIKV was an endemic disease in Africa and Asia that produced periodic outbreaks. Since then large urban epidemics have occurred in India and the virus has been detected in the Western hemisphere. In 2014, there were infections in 43 countries in the Americas involving over 1.1 million people and it is estimated that there are over 3.4 million people infected globally [131]. There is no approved vaccine. In non-human primate models, infection stimulates neutralizing antibodies as well as reactive T cells [132] and administration of a human recombinant monoclonal antibody to infected Rhesus macaques caused rapid elimination of the virus and less severe joint disease that in control treated animals [133]. Humoral antibodies primarily target the structural glycoproteins on the virions surface (E1 and E2) and these are the targets for most vaccine strategies [131]. The first vaccine tested was a formalin-inactivated derived from viruses cultured obtain from infected suckling mouse brains and later from *in vitro* cultures at Walter Reed Army Institute. More recently inactivated virus obtained directly from an infected patient and live-attenuated virus have been tested in human clinical trials and effectively induce neutralizing antibodies [131].

Unfortunately, these approaches were discontinued in 1998 due to limited funding and the unpredictable outbreaks of the diseases [134]. A positive response correlated with INF-γ, a product of activated CD4 + T cells (DTH), in an experimental mouse model [135]. Active investigation of a variety of vaccine options is now underway including virus like particles, subunit vaccines, vectored and chimeric vaccines, nucleic acid vaccines and live-attenuated vaccines [132]. Clinical trials are limited by lack of funding. Because DTH most likely is critical for a therapeutic vaccine, a recombinant BCG vaccine should be considered.

2.4.5. West Nile fever
West Nile virus is transmitted by mosquitoes from infected birds to animals, including dogs, horses, cats and humans [136,137]. About 20% of humans exposed develop fever, fatigue and weakness only, but a small number develop a severe infection that can last for months and produce severe brain damage [138]. There is no approved vaccine; the best method to prevent the spread of the disease is to avoid mosquitoes. Also, since birds are the primary reservoir for the virus, risk can be evaluated by the presence of the virus in dead birds. As with other *Flaviviridae*, after being bitten by an infected mosquito, the virus enters and replicates in dendritic cells and monocytes. These infected dendritic cells may inhibit NK cell protection and other T-cell functions [139]. There is no approved vaccine for humans, but a vaccine called ChimerivaxWNV® is under development and in stage II clinical trials [140]. Again, in addition to receptor blockade to prevent infection, consideration should be given to induction of DTH to activate infected macrophages to kill intracellular organisms.

2.4.6. Zika virus
Zika virus is a mosquito-transmitted virus in the *Flaviviridae* family of RNA viruses. It was first identified in a monkey in the ZIka forest of Uganda. Zika virus emerged as a clinically significant problem in 2015 when the virus spread to South America causing congenital malformations and microcephaly and Guillain–Barre syndrome in adults [141]. By 2018 over 800,000 cases were reported in the Americas, including the U.S. Five vaccine designs are currently under investigation: DNA vaccine, purified inactivated virus, live-attenuated virus mRNA vaccine and viral vector based [142]. There are multiple epitopes of the envelope proteins (E) that could be targets for neutralizing antibodies [143]. The vaccines include the E and PrM proteins which make up the outer protein coat of the virus virion [141]. Experimental studies demonstrate that the vaccines induce blocking antibodies as well as CD8 + T-CTL to structural proteins [143]; both are desirable for protective immunity. There is no approved vaccine and many problems of evaluation of effectiveness and possible adverse outcomes remain.

2.4.7. Typhoid fever
Typhoid fever is caused by Gram-negative bacteria in the *Salmonella* family (*Typhi* and *paratyphi*). These are found only in man and are transmitted by ingestion of contaminated food or water. The organism penetrates the small bowel epithelium and spreads by lymphatics to the blood stream. It may be treated with antibiotics but increasing incidence of drug resistant strains has made prevention by vaccination more important than previously. Untreated about 20% of those infected will die [144]. Sanitation during food preparation is the most effect way to prevent the disease. While the disease is rare in developed countries, it is particularly high in Asia and part of Africa. In the US, the vaccine is only recommended for those at high risk, such as travelers to endemic areas [145]. There are multiple immune effector mechanisms in response to infection with *S. typhi* or *S. paratyphi* infection: first, natural immunity in the form of IFN-γ producing natural killer cells and then adaptive immunity. There are antibodies in both blood and intestinal secretions to both cell surface and flagellar antigens (O, Vi, and H) with evidence of both systemic and local (Peyer’s patch) antibody production, as well as activation of CD4 + T cells. Since the organisms infect cells in the monocytic series both in the intestine (M-cells) and lymphoid tissue (monocytes) it is likely that T-cell-mediated activation of macrophages (delayed hypersensitivity) would be active. The first typhoid vaccines were developed in 1896. Since then there have been many improvements [145]. There are several safe and effective widely used vaccines: Vi capsular vaccine (*Typhim Vi®*) injected intra-muscularly; *Ty21* oral vaccine (Vivotiff®) and combined with heptatitis A and Vi polysaccharide (ViVaxin® and Hepatibrix®). The capsular polysaccharide vaccines are isolated from the *Ty2* strain of *S. typhi* and have limited T-cell responses and booster effects [146], but are effective in producing IgM antibodies. The oral vaccines do not contain Vi antigens but promote a local mucosal immune response in the GI tract with IgA antibody as well as systemic IgG antibody and T-cell activation [147]. Conjugate and live-attenuated vaccines are under development [146] and have greater potential for T-cell activation.
2.5. Cytoytic

2.5.1. Malaria

The malaria parasite, *Plasmodium falciparum*, caused 219 million infections and 435,000 deaths in 2017, essentially the same as in 2016 (212 million infections and 429 deaths [138]). These figures have been even higher. Between 2000 and 2013, the incidence fell by 30% and mortality by 47% due to preventive actions such as distribution of bed nets, indoor residual spraying and artemisinin-based therapies [138]. Future gains against malaria depend on availability of an effective vaccine [139]. This is complicated by the complex life cycle of *P. falciparum* as well as other malaria causing strains. The infective stage of *P. falciparum*, (sporozoites) is carried by the female Anopheles mosquito. Upon biting a human, the sporozoites infect hepatocytes where they multiply and develop into merozoites. These are released into the circulation and infect red blood cells, where they produce symptoms by destruction of RBCs. A small number mature to male and female gametocytes which, when taken up by the female Anopheles mosquito during a blood meal, will reproduce to form sporozoites, completing the life cycle [148]. Vaccines may be directed to the pre-erythrocyte stage, blood stage, or sexual stage. Erythrocytes lack major histocompatibility antigens and serve as a protective niche for merozoites against T-cell-mediated mechanisms but do express parasite antigens that can be recognized by antibody. Thus, antibody-mediated cytotoxicity is the major mechanism for immune defense against malaria. The antigens expressed on the infected RBCs reflect genetic variations of the organisms to avoid this immune attack. An effective vaccine must elicit antibodies to a variety of possible plasmodium antigens. Vaccines for malaria have been under development for over 50 years [148]. These are directed to the pre-erythrocyte antigens, whole organisms and blood stages.

2.5.1.1. Pre-erythrocyte vaccines. Vaccines for the liver stage of infection would prevent malaria infection so that merozoites are not produced to then infect erythrocytes. This vaccine is directed to the circumsporite surface protein (CAP). The vaccine consists of a central repeat region (RO), T-cell epitopes (T) and a carrier matrix of HBV surface antigen (HBsAg) as a fusion protein co-expressed in *Accharamyces cerevisiae* along with free HBsAg [150]. This is combined with an adjuvant from an oil-and water-emulsion of monophosphoryl lipid A and a saponin derived from the South America soap bark tree Quillaia Saponaria. If effective the immune response would induce T-cell cytotoxicity to kill infected hepatocytes. In field tests in Africa this proved up to 50% effective with limited side effects [148]. The vaccine is about 30% effective in preventing infection in infants and in April of 2019 has been approved for this use. The rationale is that even though the effectiveness is low, because of the large number of infections in newborns and infants, even this effectiveness may be significant in lowering infection rates. However, questions regarding the influence of this vaccine on shift of infection to other strains remain. Many other pre-erythrocyte antigens and adjuvants are under study [138].

2.5.1.2. Whole organisms. This approach is based on the finding that sporozoites killed by irradiation could result in sterile protection after homologous infection in about half of immunized adults (for summary of result [148]). A more promising approach now in field trials is to combine an attenuated infectious parasite with deletion of three genes that arrests at a late stage in the liver with an antimalarial drug.

2.5.1.3. Blood-stage vaccines. These are directed to reducing clinical illness. These are directed to merozoite surface antigens and a red cell membrane protein which contributes to RBC invasion. When combined with a potent liposomal adjuvant this was more that 60% effective in one test in children, but not in another test in adults [148]. Tests are ongoing with a variety of similar approaches including one for *P. vivax*. The immune mechanism is antibody-mediated cell lysis (cytotoxic).

2.6. Atopic

2.6.1. Helminths

Helminthiasis is an infection by worms including tapeworms, flukes and roundworms. These worms usually live in the GI tract but may burrow into other tissues where they induce inflammation. The eggs or the worms are found in soil and are taken in orally. The infection leads to poor development, malnutrition and anemia. During helminth infections, there are elevated serum levels of IgE, tissue eosinophilia, mastocytosis, and Th2 helper cells. These cells produce IL-4, IL-5, and IL-14 and are believed to mediate protection against helminth infection [151]. Schistosome eggs in the liver or intestine induce a strong eosinophil-rich inflammatory granuloma. Other atopic reactions associated with infection are systemic anaphylactic reactions, eczema and asthma [152], especially if infected early in life [153]. The WHO estimates that over 2 billion people are infected with soil-transmitted helminthiases, including schistosomiasis, trichomadiasis, filariasis, onchocerciasis and echinococcosis [154]. These infections appear to be able to suppress an immune response through T-cell regulatory mechanisms [135]. At the present there are no effective vaccines for human helminth infections. In animal models, vaccination is successful in inducing worm expulsion [155]. Radiation attenuated larval vaccines are effective for bovine lung worm and dog hook worm. There are three problems: identifying the protective antigens, the immune effector mechanism and an immunization protocol [155,156]. There is consensus that the immune effector mechanism is atopic (Th2), but it is not clear how to direct immunogen to this pathway. This may be done by selecting the appropriate adjuvant; for example: diethylaminoethyl (DEAE) dextran [157]. Human trials are now underway.

2.6.2. Schistosomiasis

According to the WHO, *Schistosomiasis* is the third most devastating tropical disease globally, and a major cause of morbidity and mortality. Although all 5 species can be treated effectively with high doses of praziquantel [158], reinfection is frequent, and chemotherapy has limited value for prevention. Clearly, a
vaccine is needed for effective control. However, development of a vaccine for schistosomiasis has proved to be elusive and faces complications because of the complex life cycle of the worm, which includes several distinct phases. Humans are infected through the skin through fresh water contaminated with free living cercariae derived from infected snails [159,160]. Within 2 days the cercariae changes form and burrows through the dermis, enters draining blood vessels, migrates to the lungs and then to liver where they feed on red blood cells, mature and mate. Male-female worm pairs form and settle in either the portal or pelvic vessels, copulate in situ and lay eggs which are released into the stool or urine [161]. Because of the various forms that emerge during this life cycle up to 20 different potential immunizing antigens have been identified; three of which: SM14, Sh28GST, and AM-TSP-2 have entered clinical trials [162]. Since there are no specific receptor-ligand interactions and no intracellular infections, potentially neutralizing (receptor blocking) antibodies would not be expected to contribute to defense. In tissues eggs elicit a cellular reaction consisting of eosinophils, lymphocytes and granuloma formation with hyperplasia of overlying epithelium [159]. The inflammatory response includes Th2-type cytokines [163] suggesting an atopic hypersensitivity reaction with granuloma formation consistent with poorly degradable antigen. Production of antibody may result in a serum sickness-like illness known as Katayama syndrome [164]. In chronic disease Th2-type response (IL-4, IL5, and IL-13) contributes to fibrosis and morbidity [163]. It is not clear that the atopic mechanism is effective in combatting the infection. The problem is that the atopic response may be desired for expulsion of worms from the intestine, but this also contributes to chronic tissue damage to eggs in tissues. An alternative mechanism might be DTH which could effectively phagocyte eggs and larvae. As suggested for syphilis vaccine (see below), a recombinant BCG vaccine may be the best approach to induce the desired delayed hypersensitivity response for a schistosomiasis vaccine.

2.7. Immune complex

2.7.1. Staphylococcus aureus

*Staphylococcus aureus* is a Gram-negative bacterium that is an asymptomatic resident of the nasopharynx and skin. Upon invasion beyond the epidermal protective barriers, *S. aureus* causes skin and soft-tissue inflammation as well as septic arthritis, pneumonia, septicemia, toxic shock, etc. *Staphylococcus aureus* infections were once well controlled by treatment with methicillin, but drug resistant strains rapidly appeared and now methicillin-resistant *S. aureus* (MRSA) is the leading cause of nosocomial infections world wide [165]. In the U.S., there are 300,000 hospitalization for *S. aureus* per year which accounts for up to $14.5 billion in hospital costs. *Staphylococcus aureus* produces acute necrotic infection (abscess). The adaptive immune defense is antibody-mediated opsonization (immune complex reaction). Essentially all adults have antibodies to *S. aureus* surface antigens, but these do not always fix complement and do not provide protection against infection [166]. Many vaccine candidates have targeted cell surface polysaccharides or cell wall proteins that are effective in blocking receptors effective for other infections, but these have not proven to be effective in humans [167]. Two vaccines have been tested: StaphVAX® (polysaccharide-protein conjugated) and V710® (to a cell wall protein). The first proved to be ineffective and the second increased infections in a surgical population. It is the conclusion of workers in the field that single antigen vaccines will not work so that a combined vaccine to increase both opsonizing and toxin neutralizing antibodies should be developed and tested [166]. One now under development is SA4Ag®. This contains 4 antigens: the adhesion molecule ClfA, the manganese transporter MntC and antiphagocytic capsular polysaccharides 5 and 9 [168]. This is designed to elicit broad antibody and cell-mediated immunity against multiple virulence factors and results of a Phase-I trial show a robust response to all components of the vaccine [166]. Other vaccines against surface polysaccharides, proteins, and secreted toxins, as well as passive monoclonal antibodies are in various stages of development [166].

2.7.2. Lyme disease

Lyme borreliosis is a serious multisystem disease affecting the skin, joints, nervous system and heart caused by a bacterium named *Borrelia* [169]. It is spread by infected ticks of the genus *ikodes*, which is common in the North Eastern U.S. It is estimated to affect 300,000 people a year in the United States and 65,000 in Europe [170]. Lyme disease progresses in three stage [171,172]: Stage-I cutaneous rash (erythema migrans) with lympho-plasmocytic infiltrates around dermal vessels. Stage-II lympho-plasmacellular infiltrate in seen in the meninges, ganglia, peripheral nerves and in heart in patients with cardiac lesions. Stage-III hypertrophic synovitis and cutaneous lymphoid hyperplasia are also seen. Erythema migrans begins as a red macule expanding to an annular lesion from 5 to more than 60 cm within days or weeks after infection [173] often with fever, fatigue and headache. About two-thirds of untreated children develop arthritis without skin lesions and 20% neurologic manifestations (nerve palsy) with increased lymphocytes in CSF [173]. Diagnosis may be confirmed by seroconversion. Organisms in the dermis activate proteases and outer surface proteins selectively interact with endothelial cells, platelets and extracellular matrix by interactions with integrins, glycosaminoglycans, fibronectin and collagen promoting heart and joint homing and colonization [172]. The role of the immune response appears to be protective as SCID mice which lack B and T cells develop severe arthritis and carditis [171]. Initial spread of infection may be partially limited by the natural immunity. *B burgdorferi* release molecules that produce natural immunity responses which may limit the infection and enhance the adaptive immune response, but also contribute to tissue damage. The specific immune response may involve different immune mechanisms as early arthritis is characterized by neutrophil infiltration, whereas later arthritis and carditis features macrophages and T-lymphocytes suggesting a DTH reaction. The organisms are extracellular so that receptor blockade by antibody and T-CTLs are most likely not involved in protection, but antibody to outer membranes may neutralize the defense mechanism of the organisms. The organism produces a variety of biologically active molecules that could be targets for inactivation and
outer membrane proteins, but the most promising for a vaccine are outer surface proteins OspA and OspC, which are involved in protecting the organism as it travels from the infected tick to the host [174]. A vaccine based against OspA (LYMDrix®) was introduced and tested in clinical trials to have an efficacy of 76% in adults and 100% in children. However, many recipients developed autoimmune and other side effects, and although the CDC found no connection between the vaccine and the complaints, subsequent law suits forced the vaccine to be withdrawn. In April 2019, congress provided NIH with $6 million for prevention proposals for Lyme disease [175]. The best prevention is avoidance of ticks and removal from the skin of any ticks as soon as found. Antibiotics given early after infection can prevent development of symptoms.

2.8. T-CTL

2.8.1. Human papilloma virus (HPV)

HPV is one of most common sexually transmitted infections. Most HPV infections are symptom-free and resolve spontaneously [176]. There are over 100 HPV types, low-risk HPV types produce genital warts, whereas high-risk types cause cervical cancer [177]. It is estimated that 5% of all cancers world-wide are related to HPV infections [178], including cervical, penile, anal, and oropharyngeal cancers and that up to 20% of men carry one or more of the pathogenic HPV types [179]. In 2006 the FDA licensed the first HPV vaccine directed to HPV types 6, 11, 16 and 18 with 2 doses beginning between age 11 and 15 for girls. The quadrivalent vaccine is effective in preventing genital HPV infection to these types. More recently a vaccine against 9 of the most common HPV types has been developed [157]. The vaccine is given as an intramuscular injection. HPV is transmitted directly into the epithelium infecting the basal (stem) cells to produce cervical cancer. The virus cannot infect intact epithelium but is able to bind to basal cells through micro-abrasions or other epithelial trauma [180]. The infection then prevents maturation of the basal cells so that continued proliferation and loss of differentiation causes cancer [181]. Since viral contact is directly to the epithelium, humoral antibody cannot block viral cell interactions. Also, since the infection is limited to epithelial cells, immune reactivity to the infected cells must be T-cell-mediated cytotoxicity. Humoral antibody or T-cell-mediated DTH are active in the dermis and not in the epithelium. Thus, consideration should be given to intraepithelial epithelial inoculation with a vaccinia virus vectored vaccine to induce T-CTL to infected cervical epithelium [81].

2.9. Delayed type hypersensitivity (DTH)

2.9.1. Tuberculosis and leprosy

Tuberculosis is arguably the deadliest infection of humans; over the last 200 years it has killed one billion people. It currently has more victims than almost all other infectious diseases put together [182]. The WHO estimates that in 2015 10 million people developed TB with 1.3 million deaths in HIV negative individuals and an additional 300,000 in those with HIV [183]. The infection rate and deaths are much higher in developing countries than in high-income countries with increasing cases caused by drug resistant strains.

About 23% of the world’s population has latent TB. The protective response to infection with *Mycobacterium tuberculosis* is macrophage activation by immune reactive CD4+ T-cells (DTH). *Mycobacterium tuberculosis* infects macrophages. TB specific CD4 + T cells can recognize the TB antigens on macrophages and release cytokines that activate macrophages to kill the organisms. Unfortunately, this approach may not be effective or there may be large numbers of organisms that cannot be removed by the macrophages. If the organisms and infected cells cannot be eliminated during the process of delayed hypersensitivity, surrounding macrophages are attracted and form a barrier around the infected tissue known (granuloma). Although the granuloma ‘walls off’ the infected cells; with age the granuloma may break down allowing residual viable organisms to disseminate from degenerating macrophages. HIV infection plays a major role is loss of this protection to tuberculosis and the reactivation rate in very high in patients with HIV.

For vaccine-induced immune protection, humoral antibody and T-cell cytotoxicity are ineffective and vaccination should be directed to producing DTH. For DTH CD4 + T cells release cytokines that activate macrophages to kill the organisms in their cytoplasm as well as arm uninfected macrophages to phagocyte and kill organisms released form infected monocytes. In 1921 a vaccine for tuberculosis was developed by the French scientists Albert Calmette and Camille Guerin (bacilli Calmette Guerin, BCG) [184]. BCG is an attenuated strain of cow tuberculosis and they thought it should do for tuberculosis what cow pox did for small pox. Immunization with BCG induced a positive DTH reaction (tuberculin test). BCG has the capacity to limit military TB in infants, but it fails to prevent TB in older age groups [184] and is pathogenic in immune compromised individuals such as HIV patients [183]. For this reason, BCG is not recommended for use in the US, but is recommended in other countries. Because of the limits of BCG vaccination, alternative vaccines have been prepared and tested [185]. These include *M. tuberculosis* antigens in subunit vaccines, adjuvants, viral vectors, including modified vaccinia, adenovirus and influenza virus, inactivated mycobacterial organisms and living vaccines including modified BCG and *M. tuberculosis*. None of these has yet been proved to be effective in clinical trials, but if the objective is to induce effective DTH, then the last approach (altered BCG or *M. tuberculosis*) will most likely be effective [186].

Leprosy has a similar pathogenesis as tuberculosis with bacilli infecting macrophages. The immune response in leprosy determines the outcome of the infection. If there is a predominant antibody response there is a progressive disease with disfiguration (Lepromatous Leprosy); if the response is predominantly DTH, organized granulomas will limit the progression of the disease (Tuberculoid Leprosy) [187]. The BCG
vaccine is 40% (1 dose) to 60% (2 doses) in preventing Leprosy 65 [188] and is recommended by the WHO for use in countries with high incidence of TB and leprosy [183].

2.9.2. Syphilis

Syphilis is a sexually transmitted disease caused by spirochete Treponema pallidum. Historically the high incidence of syphilis declined because of public health measures and the introduction of penicillin, to which T. pallidum is very sensitive. Currently it is still distributed world-wide with an estimated global burden of 18 million and 5.6 million new cases per year. It is very high in low income countries and in the United States it has been sharply rising in men who have sex with men and in newborns of infected mothers (congenital syphilis) [189]. It has been estimated that congenital syphilis is responsible for up to 9.5 million years of life lost [190]. The syphilis spirochete literally swims in the extracellular fluid of infected tissues; it does not react with any tissue cells [191]. At the site of contact, about two weeks after infection, there is a firm raised inflammatory reaction characterized by central necrosis, called a chancre. This is the result of a delayed hypersensitivity reaction characterized by lymphocytic infiltration followed by an influx of macrophages that phagocytose and destroy the invading T. pallidum [191,192]. This is evidence of a strong CD4 + T-cell immunity and after healing of the chancre there is strong resistance to re-infection (chancre immunity). However, in about one-third of infected humans T. pallidum organisms have spread to other sites where they are protected from immunity and cause chronic inflammation and tissue damage (secondary and tertiary syphilis). Clearly, a vaccine for syphilis would be very effective in reducing the disease in high-risk patients [189]. There is presently no vaccine for syphilis and to the best of the writer’s knowledge, no efforts to produce one [193]. Potential immunogens belong to the T. pallidum repeat (Tpr) family of proteins and adhesion proteins on T. pallidum. Since the protective immune response in syphilis is delayed hypersensitivity (DTH), whatever immunogen is selected it should be constructed to induce DTH [191]. For this goal recombinants of Bacilli-Calmette–Guerin with DNA sequences coding Tpr or adhesion proteins is recommended [192,193].

2.9.3. Candidiasis

Candidiasis is a fungal infection commonly of the mouth (Thrush) or vagina (Yeast infection), and systemically in immunocompromised individuals. Thrush refers to the gross appearance of involvement of the tongue with white coating that resembles the breast feathers on the bird, Thrush. Although there are more than 150 types of Candida only about 20 can cause infection, by far the most common is Candida albicans. Individuals at high risk of infection are babies less than 6 months old (6%), and those receiving chemotherapy for cancer or with AIDS (20%). In healthy individuals, Candida is part of the normal flora of skin and intestine but may become a localized infection of the skin, fingernails and toenails, or mucous membranes of the oral cavity or genitalia [194]. Candidemia is a blood infection that even with treatment has a mortality rate of 50%. This condition is on the rise, especially in immunocompromised patients with an incidence of about 400,000 cases per year in the US. It is treated by antifungal medications, such as nystatin, etc. There is no vaccine but there is general agreement that a vaccine is needed not only because of the increased incidence, but also because of high hospitalization costs and increasing drug resistance [194]. Strategies that have been used to producing a vaccine for C. albicans include live-attenuated strains, recombinant proteins, and glycoproteins, including use of water-in-oil emulsions [195]. Although promising results have been obtained in experimental animals, no vaccine has advanced to clinical trials. There are several major problems. First, the organism is extracellular, and infection does not involve binding or entry into cells, thus many of the immune effector mechanisms applied to other infections, such as receptor blockade and T-CTL, are not applicable to Candida. Then, the organism transitions among yeast, pseudo-hyphal and hyphal forms, as well as a heritable white to opaque phenotypic switch, providing a moving target for the immune response [195]. In addition, normal residence in the intestine may lead to tolerance to a third independent epigenetic state [195]. It appears that the most applicable immune effector mechanism is delayed hypersensitivity (T-cell-mediated macrophage activation). As with Leprosy, an antibody dominated immune response is associated with progressive disease, whereas macrophage activation limits the disease. Thus, vaccination should be directed to DTH. This poses a problem for Candidiasis. For DTH the method of choice would be recombinant BCG as proposed for a syphilis vaccine. However, the immunocompromised patients for whom a vaccine is most appropriate, not only have a limited ability to produce DTH, but also are susceptible to BCGosis, an active infection with BCG. In any case, it does not appear that there will be a vaccine for candidiasis in the foreseeable future.

2.10. Alzheimer’s disease

Vaccination for Alzheimer’s disease (AD) poses unique and challenging problems as the target is not an infectious agent, but an autologous protein. AD is a progressive loss of memory and cognitive thinking due to the accumulation of misfolded protein breakdown products in neurons in the brain [196]. There are about 30 million people in the world with this disease (6% of all people over 65 years of age) with an estimated 2 million deaths per year [197]. The abnormally folded proteins are tau and amyloid beta protein (Aβ) [198–200]. Accumulation of tau produces aggregates of neurofibrillary tangles, whereas accumulation of small peptides Aβ results in amyloid plaques [198]. These accumulations are believed to cause loss of neural function and the symptoms of AD. The rational for vaccination is to prevent AD is to produce antibodies that will block the accumulation by preventing the peptides from reaching the neurons. The rational for treatment of AD is to produce antibodies (or passively transfer antibodies) that will remove the accumulations from the affected neurons [201]. Some antibodies are not able to enter neurons and work in the extracellular compartment where they may sequester tau aggregates, interfere with assembly or promote microglial phagocytosis [200]. Antibodies that can enter neurons bind to tau aggregates
within the endosome/lysosome and promote disassembly, leading to enhanced degradation by lysosomal enzymes; sequester tau assemblies in the cytosol; or promote proteasomal degradation via E3 ubiquitin-protein ligase binding [200].

For active immunization AAADvac-1 is for Tau epitope 294–305 and ACI-35 is to PSer396,404. These have proven to be safe in Phase-I clinical trials and have advanced to Phase-II trials Seven different antibodies are in Phase-I or -II trails of passive immunity [201,202]. Many variations of antibody specificity (tau epitopes), isotope, charge, fragments, etc., have been tested in transgenic mouse models of AD with some success [202–204]. As the trials are early and still in progress there has been no evidence of efficacy in humans.

A similar approach is proposed for prevention and treatment of Parkinson’s disease (PD) [199]. In PD accumulation of aggregates of a-synuclein in neurons produces PD symptoms. Active and passive immunization is proposed to inhibit aggregation promote intracellular degradation by enhancing autophagy and lysosomal degradation [205].

3. Expert opinion

The reduction in morbidity and mortality associated with vaccine-preventable diseases in the United States is ‘one of the greatest public health achievements of the first decade of the 21st century’ [206]. Many believe that this is an underestimate. Much of the success of vaccination has been by trial and error. Understanding of antigens, conjugates, adjuvants, mode and route of administration, and testing have progressed so that vaccines may be designed to elicit induction of the immune effector mechanism(s) that will be most effective against the infection. The protective and therapeutic effector mechanisms are listed in Table 2. The first mechanism ‘inactivation of biologically active molecules’ is effective against symptoms that are caused by toxins; for example, tetanus and diphtheria toxins without enlisting any other mechanisms. For pertussis and cholera toxoid vaccines are vital, but other immune effector mechanisms may be required for full protection because inactivation of the toxin alone is not always up to the task of preventing the disease by itself. Receptor blockade is another neutralization mechanism. This mechanism is the most common mechanism and is effective for infectious agents, such as viruses, that live inside cells, by preventing the infectious virus from entering the target cells. For viruses that infect epithelial cells T-cell-mediated cytotoxicity may critically enhance the therapeutic effect of vaccination. For viruses that infect epithelial cells T-cell-mediated cytotoxicity may critically enhance the therapeutic effect of vaccination. For viruses that infect epithelial cells T-cell-mediated cytotoxicity may critically enhance the therapeutic effect of vaccination. 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Activated macrophages actively phagocytose and lyse T. pallidum in the interstitial fluid.

3.1. Five-year view

Progress can be expected in two approaches: more widespread use of safe and effective vaccines, such as those for diphtheria, tetanus, cholera, pneumococcus, measles, varicella, Ebola virus, rotavirus, hepatitis A and B, polio, Japanese encephalitis; and development of new approaches for vaccination that are directed to the appropriate immune effector mechanism(s). The first approach is hampered by ‘cognitive dissonance’. Many have religious or other irrational preconceived concepts that vaccines are dangerous (cause autism, induce sterility, etc.). Even when presented with convincing data that vaccines are safe and effective, they chose for themselves and family members not to be vaccinated. This effectively prevents herd immunity and allows diseases that should be eliminated to reappear in epidemics (measles in 2019). Use of adjuvants or conjugate vaccines may provide improved vaccines for Pertussis, Anthrax; Haemophilus influenzae, Meningococcus and S. aureus. Use of adenovirus vectors, such as Vaccinia virus, to induce TCTL could be effective for Influenza, mumps virus, HIV, Hepatitis C, Chagas disease, HPV, and malaria [207]. For yellow fever, Dengue, West Nile virus, Typhoid, Lyme disease, Helminths, TB, Leprosy, and Syphilis vaccines that induce DTH may be more effective than present vaccines. The problem is that a consistent, safe and effective way to induce DTH has not been identified [207]. The present state of the art indicates that recombinant BCG may be worth trying [192], but more effective approaches may be necessary. Adjuvants including bacterial products in water and oil emulsions are known to induce DTH in experimental animals [208], and DTH may precede an antibody response (CD4 + T-cell priming) when adjuvants are used [209]. Unfortunately, the DTH adjuvants used produce local inflammatory reactions unacceptable for human or even animal use. New approaches to induce DTH should be a high priority for vaccine development [210].

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References

Papers of special note have been highlighted as either of interest (-) or of considerable interest (--) to readers.

   -- Best source for information on immune effector mechanisms.

   • First classification of immune mechanisms.

   -- Extensive review of vaccines.

   • Papers of special note have been highlighted as either of interest (-) or of considerable interest (--) to readers.

   • Massive data base.

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