

## IMMUNITY AND VACCINES

**SOME PUBLIC HEALTH.** The world's population grew slowly up to the Industrial Revolution (mid 18c), and then began its current exponential increase (about 1.13% per year). The major causes of death had been smallpox, typhoid, typhus, cholera, tuberculosis, childbed fever, whatever the Black Death was, and plague. Why did these diseases begin to decline? By far the most important reason was the improvement of public sanitation: clean water, closed sewers and sewage treatment, and less crowded housing. Immunization was introduced by Jenner in 1796, and much advanced by Pasteur, von Behring, and Kitasato in the late 19c. Antibiotics arrived in the 1940s. This convinced some people that infectious disease would soon be a thing of the past, because it seemed that we could immunize against or treat any infection.

“One can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, **the virtual elimination of the infectious diseases as a significant factor in social life.**” Nobel Prize winner F. Macfarlane Burnet, 1962

But they didn't anticipate AIDS, which makes demands on vaccine design beyond our current skills. Nor did they realize that the misuse of antibiotics would lead to epidemics of drug-resistant infections, including MRSA and MDR/EDR<sup>1</sup> tuberculosis. Possibly worse, we have so altered our relationships with wild animals and birds that we are exposed to unprecedented infectious agents, like H5N1 and 2009/H1N1 flu, Ebola, Zika, SARS, and West Nile viruses. Polio, which should have been eradicated by now, persists in places of political or military turmoil.

**KINDS OF IMMUNITY.** It will help your understanding to consider that immunity may be ► naturally or artificially induced, and active or passive.

**Natural, active:** this is the usual immunity that results from real-life exposure to a pathogen. It is not necessary to know that you've been ill; for example, 60% of mumps contacts will become immune to mumps without getting the disease. Natural is usually the most long-lasting immunity, and some people believe that getting sick ('testing in the fire') is the best thing for a child; but if there is a vaccine, it is bad practice to allow a child to contract a disease that might leave permanent damage or even be fatal.

**Natural, passive:** passive means that you are enjoying the products of someone else's immune response, and natural means it's the way Ma Nature designed it. In humans, the fetus gets IgG passively across the placenta from its mother, and the baby gets IgA in mother's milk if she nurses it (this is, of course, one of the best reasons for nursing babies rather than feeding them on cows' milk or squished soybeans.) ► Nursing has been shown to protect kids from infections. When immunogens are present in the mother's gut, antibodies to them appear in her milk.

**Artificial, active:** immunity resulting from intentional immunization with vaccines, toxoids, or other antigenic preparations. The 'dirtier' a vaccine is, that is, the more complex the mixture of molecules in it, the more likely it is to cause unpleasant side effects. Thus killed whole bacteria (the former pertussis and typhoid vaccines) were painful to receive, and often caused fever or worse. The relatively newer, *acellular* pertussis vaccine (called aP instead of P) has admirably few side effects; the downside is it isn't as good a vaccine, and badly needs to be reformulated. Tetanus and diphtheria toxoids, being relatively pure, are innocuous. With viruses, it has repeatedly been shown that 'live'<sup>2</sup> (infectious but *attenuated*, that is, grown in culture or animals long enough that it is still immunogenic but no longer causes disease) vaccines provide better immunity than do killed preparations. Why, do you think?

<sup>1</sup> Multiply/Extensively/Drug Resistant.

<sup>2</sup> Viruses are not really alive, they are *infectious information*. In fact, they are chemical structures; the formula for poliovirus is C<sub>332,652</sub>H<sub>492,388</sub>N<sub>98,245</sub>O<sub>131,196</sub>P<sub>7,501</sub>S<sub>2,340</sub>.

There is always some concern that an infectious vaccine may revert to a virulent condition. We actually only have one example of that, though: the live polio vaccine can get shed by recently-vaccinated kids and pass to new hosts if the water system is not world-class. It has caused some cases of polio-like disease in immunosuppressed (including malnourished) children. With new DNA technology it is possible to tailor the genome of viruses so that the virulence factor genes are missing or so altered that reversion to virulence is impossible. The ultimate in vaccines may very well be the products of specific genes, cloned in yeast or bacteria or totally synthetic, completely purified and then if necessary coupled to a powerful immunogenic carrier. For example, newborns now get a synthetic peptide vaccine containing the epitopes that immunize T cells best against the hepatitis B virus, grown by recombinant DNA technology in yeast cells.

**Artificial, passive:** the use of immune serum or antibodies to protect a patient at risk from a particular disease. Before the antibiotic era, horse antiserum against pneumococci (*Streptococcus pneumoniae*) was given to pneumonia patients, and horse diphtheria antiserum to diphtheria patients, and it often<sup>3</sup> pulled them through<sup>4</sup>. We still use animal antisera to snake venom and botulinum. But: there is a risk of serum sickness (see Type III Immunopathology). Now when possible we use human antisera: tetanus, rabies, hepatitis, and chicken pox are most common; rattlesnake is available. They must be screened for hepatitis and AIDS viruses, of course, but we can't screen for everything. **Remember:** Unless it has been specifically (and expensively!) formulated for intravenous use, IgG will aggregate in the bottle; aggregated IgG activates complement; complement is anaphylatoxic; *so never give these human immune globulins intravenously.* (Curiously, horse and sheep antibodies activate human complement only poorly, so these antivenins *can* be given i.v.)

### SOME SPECIFIC EXAMPLES.

**Tetanus** immunization offers close to 100% protection. There is only one form of toxin, and the toxin is the bacterium's only virulence factor, so an immunogen made up of inactivated toxin (**toxoid**) will almost always be effective. In contrast, with many other organisms such as the influenza and AIDS viruses there is substantial antigenic variability. Protection against tetanus is good for 10, perhaps 20 years. There are no important side effects, and tetanus toxoid costs (in bulk to the World Health Organization) about 35 cents a dose. Compare this to treatment: antibiotics are only partially effective, since it's the release of toxin, not the growth of bacteria, that must be stopped immediately. Antitoxin can be used; but there isn't enough human TIG (tetanus immune globulin) to treat the 500,000 cases that occur annually worldwide, and the animal antitoxins, while effective, can readily cause serum sickness. Once the disease is established (25 cases/year in the U.S.) it may require surgery, intensive care units, hyperbaric chambers, and so on, at an average cost of \$250,000. Because of crippling, rarely justified lawsuits and a low profit margin, tetanus toxoid has often been in short supply. Worldwide, 180,000 infants die annually from neonatal tetanus, most of this could be prevented by antenatal immunization of the mothers.

**ASK YOURSELF:** You are on call in the emergency room, and a farmer is brought in; he stepped on a rusty nail four days ago, and his teeth look a bit clenched. How do you treat him? Tetanus toxin? Tetanus toxoid? Horse antitoxin? Human antitoxin? Why did you make those choices?

<sup>3</sup> Pneumococcal bacteremia mortality: Untreated, 85%; treated with antiserum, 50%; treated with antibiotics, 10%.

<sup>4</sup> You can read the story of Balto, the dog who led the sled team that brought the diphtheria antiserum to Nome (and thus led to the Iditarod sled race) in **Supplementary Material: Balto**. Dramatic stuff.

**Smallpox** may have been the worst infectious disease ever. There is no good treatment—the last known case in the world, a British medical photographer, died in spite of the most sophisticated care. This disease has been *eliminated* by immunization. We can hope to eliminate an infectious disease if (a) its only host is the human, and (b) there is no environmental reservoir for it. So, measles, yes; rabies, no.

**Rabies** active immunization is possible with a vaccine grown on human diploid cells (HDCV), which has admirably few side effects. If someone has been bitten by an animal known to be rabid, passive immunization with human rabies immune globulin is imperative, as well as active immunization (4 shots of HDCV). Because the onset is slow, ► this is one of the only diseases where immunization may begin *after* exposure. Otherwise, the case fatality rate is essentially 100%. You can read about the panic in a Montana school in a supplementary file on the web site.

**Diphtheria** toxoid costs about thirty cents and immunity lasts years. Antibiotic treatment of a case costs about \$30; not bad, except if your annual income is about \$60, as in some parts of the world. In the former Soviet Union, immunization was largely discontinued for reasons of cost in 1990. 150,000 cases of diphtheria resulted, with 5,000 deaths by 1998.

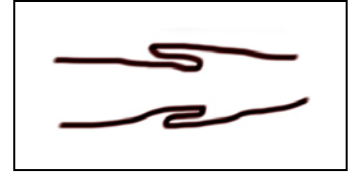
**Pertussis** is whooping cough. Acellular pertussis (aP) vaccine is 10 times safer than the old form. In 1940, before the vaccine, we had 250,000 cases of whooping cough in the U.S., and 7,000 deaths. *7,000!* Please mention these numbers when parents are reluctant to immunize their child, and warn them that recent research shows *no* herd immunity protection for unimmunized children, who have a 20-fold increase of risk. Incidence is slowly rising worldwide; the reasons are being studied, but new pertussis strains are likely an important reason (the vaccine is based on strains prevalent in 1950).

**Measles** was nearly gone from the USA; that happened when we got the immunization rate up to about 90%. In Ireland it slipped to 70%, and they had 1,600 cases in 2000, with 350 hospitalizations and 3 deaths. In that same year, the U.S., with 80 times more people, but a national measles immunization rate near 90%, had 100 cases. There were 667 reported cases in 2014, though, the highest number since endemic measles was eliminated. Worldwide, 250,000 people still die each year of measles, almost all of them children. There were over 25,000 cases in Western Europe in 2011, 2012, and 2013. With a general tightening of immunization rules, cases fell to 3800 in 2014. The UK's 137 cases in 2014 were mostly in adolescents who had missed their measles immunization during the Wakefield-induced panic.

Sanitation and immunization can prevent an incredible amount of illness and death. It is here that health care professionals, by making sure their patients are well educated, can save hundreds and hundreds of lives. And yet, immunization is under constant attack by people who don't understand science. Why?? *Nothing in medicine makes better sense than preventing an illness.*

**HARNESSING HELP WITH CONJUGATE VACCINES.** ► The major immunogens for *Hemophilus*, *Streptococcus*, and *Neisseria* are found in their capsular carbohydrates. As you know, responses to carbohydrate epitopes are T-independent, and consist mostly of IgM. But IgM does not penetrate well into tissues, and alone it provides insufficient immunity to actual infection. *Hemophilus influenzae* type b, a bacterium, causes childhood meningitis. The original *Hib* vaccine, a complex carbohydrate, was bad at immunizing these kids; they made only some IgM and failed to switch to IgG. So the carbohydrate was coupled to a protein 'carrier' to which Tfh cells could respond (tetanus or diphtheria toxoid, usually) and thus focus their help on the B cells responding to the attached carbohydrate. ► These **conjugate vaccines** are highly effective in babies (and in the elderly, too). Conjugate *Streptococcus pneumoniae* multivalent vaccines and *Neisseria meningitidis* vaccines are very effective, too.

**VACCINES DO HAVE PROBLEMS.** RotaShield<sup>®</sup>, a tetravalent vaccine against rotavirus, the most common cause of severe diarrhea in the US and a killer of 500,000 children annually worldwide, was released in the US in 1998. Within a year, cases of intussusception were reported in children receiving this live, attenuated vaccine, and the CDC recommended its withdrawal. Intussusception is the telescoping of a segment of bowel. The risk of this phenomenon is that part of the wall will lose its blood supply due to compression of vessels and become necrotic, leading to peritonitis. At first it seemed incomprehensible that this physical symptom could be associated with an immunization, but it is possible that the vaccine in some children (about 1:10,000) caused hypertrophy of Peyer's patches due to an unusually vigorous immune response, and that normal peristaltic traction on these enlarged mucosal nodes pulled the bowel along and into itself. There are 2 new, safer rotavirus vaccines: RotaTeq<sup>®</sup> and RotaRix<sup>®</sup>.



Influenza vaccination is rarely (0.8 cases/1 million doses) associated with Guillain-Barré Syndrome; this may be coincidental rather than causal. One batch of the 2009 pandemic flu vaccine has been associated with increased risk (6x) of narcolepsy, which developed in 1300 of the 30 million people immunized. In mid-2016 the CDC was told that the nasal flu vaccine, FluMist, had been ineffective for the past 3 years.

**ADJUVANTS.** These are substances added to vaccines to make them more immunogenic. ► They all seem to work by causing an innate immune response, which then leads to a more effective adaptive response. The most common approved adjuvant in the US is alum, a hydrated potassium aluminum sulfate, and related aluminum compounds. It mimics certain PAMPs. It stimulates effective antibody responses (it is in tetanus and DTaP vaccines) but not very impressive T cell responses, so it seems mostly to generate DC that drive Tfh. In Europe several firms make approved, effective adjuvants, commonly based on the steroid precursor squalene, which activate both T and B responses. The only other approved adjuvant is lipid A, part of endotoxin. It's in Cervarix<sup>®</sup>, one of the HPV vaccines.

**HERD IMMUNITY.** The proportion of a given population that has immunity against a particular infection; it is commonly expressed as a percentage. **Herd effect** is the decrease in infection rate in the non-immune part of the herd, as a consequence of herd immunity. Two probabilities are in play: the chance that a susceptible, non-immune member of the herd will contact an infectious member (which decreases as herd immunity increases), and the chance that if such a contact occurs, disease will be transmitted (the **infectivity** of the disease organism). So diseases with a high infectivity, like measles, require high herd immunity (about 95%) to achieve a herd effect. Less infectious diseases require proportionately lower herd immunity. Obviously, for some diseases we immunize against, like tetanus, which are not passed from person to person, the concept of herd effect is irrelevant. But for smallpox, which existed only in the human species and passed person-to-person, a high enough level of herd immunity left the organism with nobody new to infect, and it died out. Not without a heroic effort: In 1967, the year the World Health Organization launched the Intensified Smallpox Eradication Programme, 10-15 million people were infected, and 2 million died. By a policy of mass vaccination and isolation of infected individuals, the disease could be declared extinct in 1997. As the director-general of WHO said, it was 'a triumph of management, not of medicine.'

**GENERAL PRINCIPLES OF BACTERIAL IMMUNITY.** ► Bacteria that live extracellularly—most of the high-grade pathogens—are combated principally by antibody. They can be blocked from attachment to mucous membranes by IgA, and once in the body they are opsonized by plasma antibody and complement. Some are controlled by lysis by C9, notably *Neisseria gonorrhoeae*; therefore people deficient in complement components need to make friends very carefully, because disseminated gonococcus infection is no fun. ► Intracellular bacteria—*Mycobacteria*, *Listeria*, *Brucella*—which get into macrophages and can survive there, are killed if the macrophage can be activated by Th1 cells. There is a TB vaccine—BCG—but it's not, at least in the USA, completely effective in producing the

Th1 and CTL immunity that is essential for protection and recovery from tuberculosis, so though it's used worldwide, it hasn't become popular here.

The complete US childhood & adult immunization schedules can be found on line at <http://www.cdc.gov/vaccines/schedules/index.html>

**VIRAL IMMUNITY.** Here is a very important general principle: ► **Humoral immunity may prevent a viral illness, but once ill, T cell immunity is necessary for recovery.**

Viruses usually enter the body through the nasopharynx, genitourinary, or GI tract. Many will establish a local infection at the site of entry; the patient may not even be aware of it. At this site the virus will multiply, and then it will spread itself throughout the body, usually by the blood. It then finds its specific targets—cells to which it can bind by surface receptors—and invades them, producing the definitive disease. Now: ► knowing this scenario, we can infer a lot about the role of the immune system. First: local immunity on the surface that is being invaded may prevent the invasion; this means secretory IgA. This is why the **Sabin** (attenuated, live) oral polio vaccine (OPV) is so effective; people immunized with it have high levels of IgA in their secretions and do not get colonized by the real virus. And recently-immunized children shed virus, which can immunize susceptible contacts. OPV was discontinued in the USA in 2000.<sup>5</sup> If the virus should slip past the IgA first line of defense, it can next be stopped during its blood-borne phase, and IgG antibody will do that. That's the kind of antibody made to **Salk** (killed, injected) polio vaccine. So good levels of viral antibody in the serum will, for many but not all viruses, indicate that the patient is not susceptible to that virus; that's why we want women in the childbearing years to have effective titers of antibody to rubella (German measles) virus.

If there is no antibody, then the virus infects cells and can alter their function or kill them. ► Now a T cell response is required. Many cells have TLR receptors for viral RNA and make dendritic-cell stimulating cytokines and chemokines in response. Dendritic cells pick up debris and free virus, and process them to peptides. They are presented on Class II MHC to Th1 cells, and cross-presented on Class I to CTL which, with Th1 help, become activated killers.

Viruses in infected body cells make early products like glycoproteins, and peptides from them are shown at the cell surface on Class I before complete viral particles have been assembled. These cells are then recognized by CTL (remember—CTL are specific for foreign antigen + MHC Class I). In this way the infected cell is killed in the viral eclipse phase, that is, the time between the virus entering the cell and the time new infectious virus particles can be made.

To summarize: ► antibody may prevent a virus from ever establishing an infection in the host. Once infection has taken place, it will be necessary to kill the infected cells before virus can multiply within them; and that is the role of T cells. Viruses which don't have to appear in the blood or lymph, or go latent and express few proteins, are very hard for the immune system to deal with. Such a virus is herpes simplex type I, which hides in your trigeminal ganglion cells out of reach of antibody or T cells, and occasionally strolls down the nerve axon to get to your lip or wherever. There it does come out of the cells, and part of the pathology of a herpes lesion is T cell-mediated immunity.

**ANTIBODY TITERS IN DIAGNOSIS.** **Titer**, incidentally, is the reciprocal of the maximal dilution of a patient's serum that is still positive in some defined test. Let's look at German measles as an example of the usefulness of measuring antibody titers in diagnosis of viral illness. This is an important

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<sup>5</sup> This policy was implemented to eliminate the risk for vaccine-associated paralytic poliomyelitis, a rare condition that has been associated with use of the live oral poliovirus vaccine (OPV). Since 1999, no OPV has been distributed in the United States.

disease, because intrauterine infection can cause fetal abnormalities. If the mother has rubella in the first eight weeks of gestation, the likelihood of fetal infection is 50%. Three-quarters of those infected will have congenital malformations. In 1964 a rubella epidemic resulted in the birth of over 20,000 infants with congenital disease. The vaccine has reduced this frequency to fewer than 25 reported cases/year.

► Babies infected *in utero* make IgM anti-rubella; determination of **IgM** anti-rubella titers in umbilical cord blood will give good evidence of congenital rubella. Since rubella is only rarely acquired in infancy, an IgM titer measured even several weeks after birth is indicative of congenital infection. Most newborns will have high IgG anti-rubella, since their mothers will have had natural rubella, or been immunized (long ago, or quite recently if their prenatal blood test indicated a low anti-rubella titer).

**ASK YOURSELF:** A baby has a 64 titer of IgG antibody against rubella at age 8 days; another has a titer of 64 at 8 months. In each case, how do you interpret it? (The minimum titer for protection is about 10, that is, the serum can be diluted 1:10 and still neutralize the virus, but not 1:20.)

In childhood or adult rubella, the rash appears 14 days after exposure. IgM rises shortly after the rash, peaks 10 days later, and falls to undetectable at about 50 days; so IgM indicates *recent* infection. (Positive antibody titers before day 14 indicate prior infection.) IgG rises a little after IgM, and stays up a long time. Note that a *rising* IgG titer is diagnostically useful, but a single elevated IgG titer is not.

**IMMUNOPARASITOLOGY.** We will talk more about immunity to parasites—protozoans, worms—in the section on Type I immunopathology, where we will see that the mechanism responsible for allergies is really the parasite immunity system.

**ORAL IMMUNOLOGY.** Dentists really have to be immunologists, too. There are many research projects designed to make vaccines against the organisms that cause caries; such vaccines could put half the dentists out of business. The other half will continue to work because the greatest cause of loss of teeth is periodontal disease; this also seems to be immunologically mediated tissue damage due to an attempted immune response to bacteria in the gingival crevice (between teeth and gums). This area is interesting, as it's a sort of no-person's-land between the mouth (rinsed by IgA and enzymes) and the body (patrolled by T cells), and lots of unsavory characters lurk there. Predictably, when people are put on immunosuppressive therapy for some illness, their periodontal disease may improve remarkably.

**DNA VACCINES.** Many groups are investigating the possibility of immunizing not with the antigen, but with DNA that codes for it. This DNA, isolated from the infectious organism and cloned into a suitable expression vector, would be injected into the skin or muscle. There it would get into cells, maintained as an episome, and be transcribed and translated. You'd become your own source of antigen, maybe for life. The advantage is speed: a vaccine like this could be available in a couple of weeks, if, say, an outbreak of Ebola fever threatened an urban area. DNA is also more stable than protein or live virus, which would be really useful in remote areas. And the antigen would be expressed on body cells, producing almost-natural, active immunity. This is interesting, but also a little worrisome. Mightn't CTL then kill the transduced cells, ending the immunity and possibly causing unexpected damage?

**ASK YOURSELF:** In the driest seasons, sub-Saharan Africa has epidemics of *Neisseria meningitidis* meningitis; in 1996, 250,000 were affected and 25,000 children died. The vaccine that Burkina Faso and Mali can afford consists of *M. meningitidis* carbohydrate. But it provides short-lasting and partial immunity, and does not work in children under about 2 years. Why? The WHO released a much better vaccine, used first in 2009 in Rwanda, at the amazing cost of 50 cents/shot; what would you guess its composition is?

## **Learning Objectives for Immunity & Vaccines**

1. Compare the roles of cell-mediated and humoral immunity in virus infections with regard to: preventing the infection; controlling spread of viruses in the body; which is responsible for recovery from disease; how each can cause immunopathology.
2. Discuss the possible roles of Th1 and CTL in recovery from virus infection.
3. Define 'local immunity' and give an example.
4. Identify those organisms against which cell-mediated immunity is most effective.
5. Identify those organisms against which humoral immunity is most effective.
6. Give an example of a human and an animal antitoxin; a toxoid; a killed virus vaccine; and a live virus vaccine. Identify the one which produces the longest-lasting immunity. Discuss possible hazards of each type of preparation.
7. State the appropriate times for immunization of children against diphtheria/pertussis/tetanus and measles. Discuss why live some viral vaccines tend to be ineffective in the very young.
8. Discuss the composition of a typical conjugate vaccine, and describe its mode of action.
9. Discuss the use of IgG and IgM antibody titers in the diagnosis of intrauterine and neonatal infections.
10. Identify the oral and parenteral (injected) polio vaccines by the names of their developers. Discuss their relative advantages and disadvantages, and note which is currently used in the USA.
11. Define 'herd immunity.'