

## 2

# Basic Vaccinology

We shall herein discuss briefly those principles of immunology, epidemiology and clinical research that are relevant to vaccinology.

### **Immunology and Vaccinology**

#### *Basic Immunology*

Immunity may be broadly classified as innate and adaptive immunity. Innate immunity comprises of the skin and mucosal barriers, phagocytes (neutrophils, monocytes and macrophages) and the natural killer (NK) cells. It comes into play immediately on entry of the pathogen and is non specific. Adaptive immunity is provided by the B lymphocytes (humoral/antibody mediated immunity) and T lymphocytes (cellular/cell mediated immunity). The innate immune system triggers the development of adaptive immunity by presenting antigens to the B lymphocytes and T lymphocytes. Adaptive immunity takes time to evolve and is pathogen specific. Humoral immunity is the principal defence mechanism against extracellular microbes and their toxins. B lymphocytes secrete antibodies that act by neutralization, complement activation or by promoting opsonophagocytosis. Antibodies are of several different types (IgG, IgM, IgA, IgD and IgE) and they differ in their structure, half life, site of action and mechanism of action. Cell mediated immunity (CMI) is the principal defence mechanism against intracellular microbes. The effectors of CMI, the T-cells are of two types. The helper T-cells secrete proteins called cytokines that stimulate the proliferation and differentiation of T-cells as well as other cells including

B lymphocytes, macrophages and NK cells. The cytotoxic T-cells act by lysing infected cells.

Active immunity is acquired through natural infection/immunization and is long lasting. Passive immunity is conferred by maternal antibodies or immunoglobulin preparations and is short lasting.

### *Types of Vaccines*

Vaccines may be broadly classified as live attenuated vaccines and killed/inactivated vaccines. Commonly used live attenuated vaccines include BCG, oral polio, measles, MMR and chicken pox vaccines. Killed vaccines may be inactivated toxins/toxoids (diphtheria/tetanus toxoids), killed organisms (whole cell pertussis vaccines) or most commonly subunit vaccines (Hib, hepatitis B, hepatitis A, typhoid, meningococcal, influenza etc). Subunit vaccines comprising only of the polysaccharide antigens are called unconjugated vaccines. Conjugation of the polysaccharide with a protein carrier significantly improves the immune response as discussed later.

### *How do Vaccines Work?*

Early protective efficacy of currently available vaccines is primarily conferred by the induction of antigen-specific antibodies that are capable of binding specifically to a toxin or a pathogen.

The role of cell mediated immunity in currently used vaccines (that have T-cell dependent antigens) is mainly by supporting antibody protection. Other less common mechanisms by which cell mediated immunity works is by cytotoxic CD8 + T lymphocytes (CTL) that may limit the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines. T-cell independent antigens (e.g., polysaccharides) do not stimulate cell mediated immunity and therefore do not produce very durable immunity. T-cell independent antigens can be converted to T-cell dependent antigens by conjugating them with proteins.

### *First Steps after Immunization*

Following injection, the vaccine antigens attract local and systemic dendritic cells, monocytes and neutrophils. These activated cells change their surface receptors and migrate along lymphatic vessels, to the draining lymph nodes where the activation of T and B lymphocytes takes place. In case of killed vaccines there is only local and unilateral lymph node activation. Conversely for live vaccines there is multifocal lymph node vaccination due to microbial replication and dissemination. Consequently the immunogenicity of killed vaccines is lower than the live vaccines; killed vaccines require adjuvants which improve the immune response by producing local inflammation and recruiting dendritic cells/monocytes to the injection site. Secondly, the site of administration of killed vaccines is of importance; the intramuscular route which is well vascularized and has a large number of patrolling dendritic cells is preferred over the subcutaneous route. The site of administration is usually of little significance for live vaccines. Finally due to focal lymph node activation, multiple killed vaccines may be administered at different sites with little immunologic interference. Immunologic interference may occur with multiple live vaccines unless they are given on the same day or at least 4 weeks apart or at different sites.

### **Immune Responses to Vaccines**

#### *Immune Response to Polysaccharide Antigens*

Bacterial (*S. pneumoniae*, *N. meningitidis*, *H. influenzae*, *S. typhi*) polysaccharide (PS) antigens are T-cell independent antigens. On being released from the injection site they reach the marginal zone of the spleen/nodes and bind to the specific Ig surface receptors of B-cells. In the absence of antigen-specific T-cell help, B-cells are activated, proliferate and differentiate in plasma cells without undergoing affinity maturation in germinal centers. The antibody response sets in 2-4 weeks following immunization, is predominantly IgM with low titers of low affinity IgG. The half life of the plasma cells is short

and antibody titers decline rapidly. Additionally the PS antigens are unable to evoke an immune response in those aged less than 2 years due to immaturity of the marginal zones. As PS antigens do not induce germinal centres, bona fide memory B cells are not elicited. Consequently, subsequent re-exposure to the same PS results in a repeat primary response that follows the same kinetics in previously vaccinated as in naïve individuals. Revaccination with certain bacterial PS — of which group C meningococcus is a prototype — may even induce lower antibody responses than the first immunization, a phenomenon referred to as hyporesponsiveness.

#### *Immune Response to Protein Antigens*

Protein antigens which include pure proteins (Hep B, Hep A, HPV, Toxoids) or conjugation of PS antigens with a protein carrier (Hib, meningo, pneumo) are T-cell dependent antigens. The initial response to these antigens is similar to PS antigens. However the antigen-specific helper T-cells that have been activated by antigen-bearing dendritic cells trigger some antigen-specific B-cells to migrate towards follicular dendritic cells (FDC's), initiating the germinal center (GC) reaction. In GC's, B-cells receive additional signals from follicular T-cells and undergo massive clonal proliferation, switch from IgM towards IgG/IgA, undergo affinity maturation and differentiate into plasma cells secreting large amounts of antigen-specific antibodies. Most of the plasma cells die at the end of germinal center reaction and thus decline in antibody levels is noted 4-8 weeks after vaccination. However a few plasma cells exit nodes/spleen and migrate to survival niches mostly located in the bone marrow, where they survive through signals provided by supporting stromal cells and this results in prolonged persistence of antibodies in the serum. Memory B-cells are generated in response to T-dependent antigens, during the GC reaction, in parallel to plasma cells. They persist there as resting cells until re exposed to their specific antigens when they readily proliferate and differentiate into plasma cells

secreting large amounts of high-affinity antibodies that may be detected in the serum within a few days after boosting.

### *Immune Response to Live Vaccines*

The live vaccines induce an immune response similar to that seen with protein vaccines. However, the take of live vaccines is not 100% with the first dose. Hence more than 1 dose is recommended with most live vaccines. Once the vaccine has been taken up, immunity is robust and lifelong or at least for several decades. This is because of continuous replication of the organism that is a constant source of the antigen. The second dose of the vaccine is therefore mostly for primary vaccine failures (no uptake of vaccine) and not for secondary vaccine failures (decline in antibodies over time).

### *Determinants of Intensity/Duration of Immune Responses*

The nature of antigen is the primary determinant; broadly speaking live vaccines are superior (exception BCG, OPV) to protein antigens which in turn are superior to polysaccharide vaccines. Adjuvants improve immune responses to inactivated vaccines. Immune response is usually better with higher antigen dose (e.g., Hepatitis B). The immune response improves with increasing number of doses and increased spacing between doses. Technically, 0, 1 and 6 months is the best immunization schedule; The first two doses are for induction and the long gap between the 2nd and 3rd dose allows for affinity maturation of B-cells and clonal selection of the fittest B-cells for booster and memory response. Extremes of age and disease conditions lower immune response.

### *Immune Memory and Need for Boosters*

As discussed earlier, immune memory is seen with live vaccines/protein antigens due to generation of memory B-cells which are activated on repeat vaccination/natural exposure. Immune memory allows one to complete an interrupted vaccine schedule without restarting the schedule.

Activation of immune memory and generation of protective antibodies usually takes 4-7 days. Diseases which have incubation periods shorter than this period such as Hib, tetanus, diphtheria and pertussis require regular boosters to maintain protective antibody levels. However diseases such as hepatitis A, hepatitis B do not need regular boosters as the long incubation period of the disease allows for activation of immune memory cells.

### *Early Life Immunization*

Though neonates and young infants need maximum protection, the immune responses in early life are suboptimal. There is interference by maternal antibodies, limited/no ability to respond to polysaccharide antigens, lower peak levels of antibodies, limited immune memory and no T-cell responses (except for BCG). Immunization schedules commencing at 2 months and having 2 months spacing between the doses are technically superior to that at 6,10 and 14 week's. However for operational reasons and for early completion of immunization and attainment of protection the 6, 10, 14 week's schedule is chosen in developing countries.

For killed vaccines such as DPT, Hib, pneumococcal and Hep B which are administered as early as birth/6 weeks the first dose acts only as priming dose while subsequent doses provide an immune response even in presence of maternal antibodies. However a booster at 15-18 months is required for durable immunity. As the age of commencement of vaccination advances the number of doses reduces (2 doses and 1 booster at 6-12 months and 1-2 doses between 12-23 months for Hib and pneumococcal vaccines).

Live vaccines are even more susceptible to maternal antibodies as compared to killed vaccines. However BCG may be given as the maternal antibodies actually enhance T-cell responses. OPV may be given as there are no maternal IgA in the gut to neutralize the virus. Furthermore measles vaccine if given at the age of 6 months (in an outbreak situation) may work by inducing T-cell immunity.

## **Epidemiology and Vaccinology**

### *Herd Immunity and Herd Effect*

Herd immunity is the proportion immune in a herd. This can be deduced from the vaccination coverage. Herd effect is the protection offered to unvaccinated members when good proportion (usually more than 85%) of the herd is vaccinated. Herd effect is due to reduced carriage of the causative microorganism by the vaccinated cohort and thus is seen only with vaccines against those diseases where humans are the only source (there is no herd effect for tetanus). An effective vaccine is a prerequisite for good herd effect; OPV in India, BCG and unconjugated polysaccharide vaccines have no herd effect.

### *Epidemiologic Shift*

This refers to an upward shift in age of infection/disease in communities with partial immunization coverage. Owing to vaccination the natural circulation of the pathogen decreases and the age of acquisition of infection advances. This is especially important for diseases like rubella, varicella and hepatitis A wherein severity of disease worsens with advancing age.

## **Vaccine Characteristics and Development**

### *Vaccine Immunogenicity*

This is the ability of a vaccine to induce antibodies. The protective threshold for most vaccines is defined. However there is often controversy about the cutoffs (pneumococcus/Hib). Levels below the limits may be protective due to other reasons such as immune memory/T-cell immunity. Bridging studies are those that look at vaccine immunogenicity but not efficacy.

### *Vaccine Efficacy*

This is the ability of the vaccine to protect an individual. It can be assessed through clinical trials, cohort studies or case control studies. It is calculated as:

$$\frac{\text{Disease in unvaccinated} - \text{Disease in vaccinated}}{\text{Disease in unvaccinated}}$$

### *Vaccine Effectiveness*

This is the ability of the vaccine to protect the community and is a sum of the vaccine efficacy and herd effect. It is revealed after a vaccine is introduced in a program.

### *Cost Effectiveness*

This is a method of economic evaluation which is carried out by mathematical modeling usually prior to introduction of a vaccine in a national program. It is expressed as cost per infections/deaths/hospitalizations prevented/life years gained.

### *Phases in Vaccine Development*

Phase 1 trials are conducted on small number of healthy human volunteers for assessing vaccine immunogenicity and safety. Phase 2 trials are conducted with a similar objective in larger number of subjects. Phase 3 trials are randomized controlled trials in large number of subjects for assessing vaccine efficacy and safety. Cost effectiveness analysis is conducted prior to introduction of vaccines in a national program. Data on vaccine effectiveness and more data on safety emerge following use of vaccines on a widespread basis in programs.

### **Conclusions**

It is hoped that some of the principles behind vaccines and vaccination schedules have become clear with the above discussion. For further details, interested readers are referred to the chapter on "Immunology of Vaccination" by Claire Anne Siegrist in the book "Vaccines", (lead editor) Stanley Plotkin, 5th Edition, 2008.