Sheet 15

Immunology

Done by | Yousef Omar

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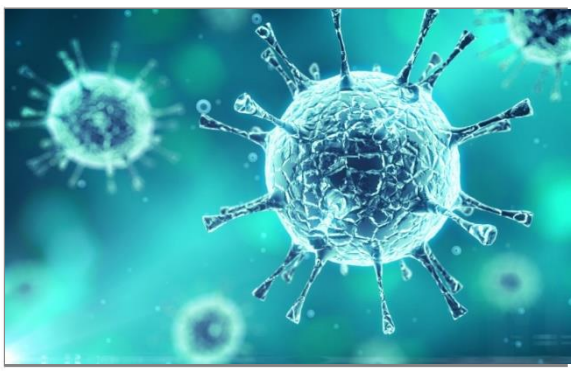


Objectives of this lecture: (Refer to slides)

- B-cell response / T-dependent B-cell response
- Vaccine types

In previous lecture, we discussed how B-cells respond to protein antigen and how B-cell require the help of T-Helper cell in order to mount a proper immune response against these antigens. Also, we have T-Independent antigens, including polysaccharides and lipids, that can activate B-cells without help of T-cells OR it can be a protein antigen, that get internalized into B-cell then present it to T-Helper cell that help improve B-cell immune function.

In the following figure:

1. Dendritic cell catches the antigen found in the tissue, process it and present it on the surface then they travel to lymph nodes (Remember Dendritic cells can also be found in lymph nodes already and catch antigens there). Then the activated dendritic cell present the antigen to T-helper cell activating it, which then moves to the follicle containing B-cells by changing the expression of its chemokine receptor (↓CCR7 ,↑CXCR5) to follow chemokines coming from the follicle.
2. Now, Within the follicle, there will be an antigen on its native form binding immunoglobulin receptor without presentation on B-cell, by this binding it gets internalized into B-cell, processed then presented on surface with MHC-II molecule. So, by this way B-cell reached a certain level of activation, but not full potential of activation. Then it changes expression of chemokine receptors to leave the follicle and meet with T-Helper cell, which help B-cell reach its full potential of activity.

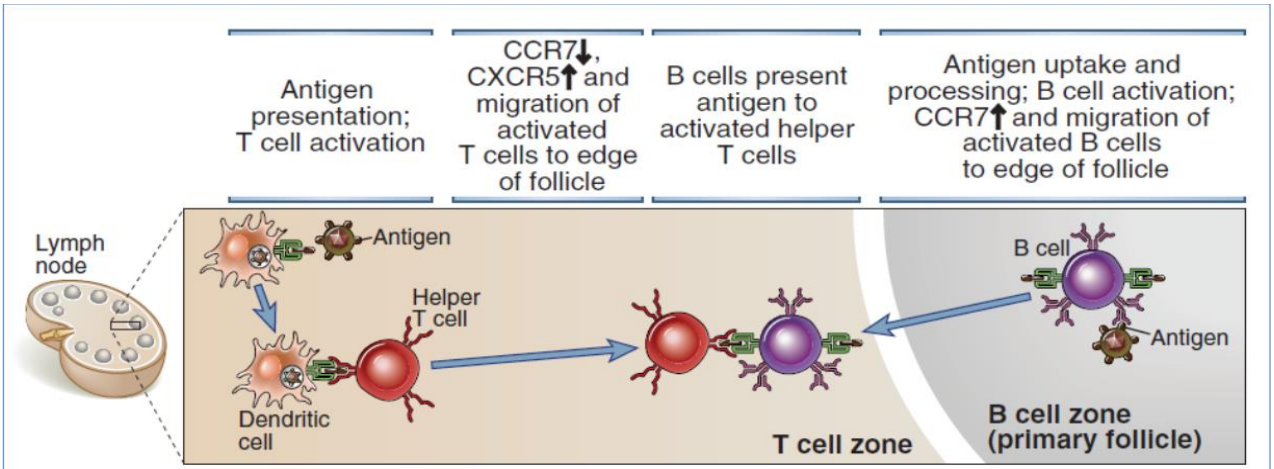


FIGURE 11–8 Migration of B cells and helper T cells and T-B interaction. Antigen-activated helper T cells and B cells move toward one another in response to chemokine signals and make contact adjacent to the edge of primary follicles. In this location, the B cell presents antigen to the T cell, and the B cell receives activating signals from the T cell.

Now, let's talk a closer look at the interaction between T-Helper cell and B-cell in the following figures:

1. So, B-cell finds a protein antigen in its native conformation, binds it and process it and then bind it to MHC-II to present it to T-Helper cell receptor(TCR), in turn T-cell become more activated and starts to express

CD40 Ligand, which binds CD40 receptor found on B-cell, which is important to fully activate B-cell. Along with that T-cells also release Cytokines, which binds to its receptor on B-cell (Cytokine receptor is expressed on B-cell surface during its early levels of activation).

2. Now, binding of CD40 and cytokine will initiate an extrafollicular focus, in which B-cells begin to proliferate and produce SHORT-lived plasma cells(they have a short life span and produce MOSTLY IgM), then these B-cells will go back to the follicle along with one of T-helper cells, so now its called follicular T-helper cell within the follicle. What happens within the follicle is the formation of the germinal center. Within the germinal center, there are activated B-cell clones (REMEMBER they all are coming from the same 1st B-cell that interacted with T-cell), Follicular T-helper cells and Follicular dendritic cells (already found within the follicle, and release cytokines to drew in B-cells). Within germinal center there will be proliferation and differentiation of B-cells to LONG-lived plasma cells and memory cells.

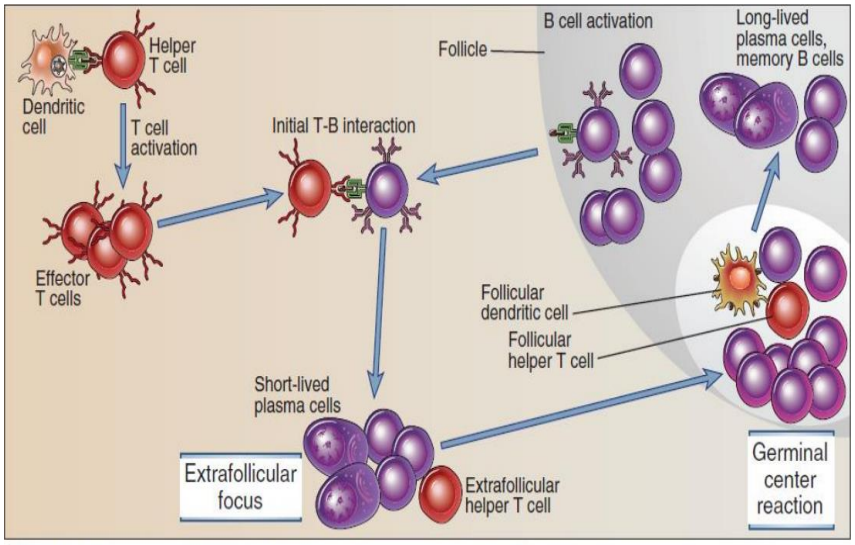
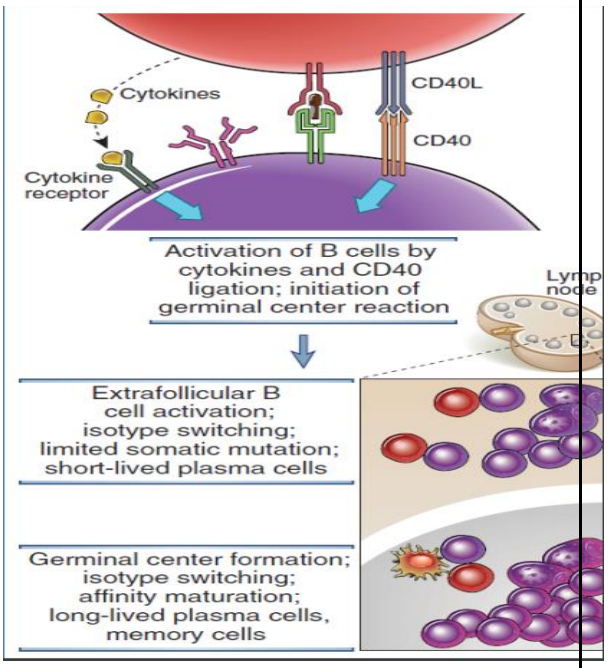


FIGURE 11–7 Sequence of events in humoral immune responses to T cell–dependent protein antigens. Immune responses are initiated by the recognition of antigens by B cells and helper T cells. The activated lymphocytes migrate toward one another and interact, resulting in B cell proliferation and differentiation. Restimulation of B cells by helper T cells in extrafollicular sites leads to early isotype switching and short-lived plasma cell generation. The late events occur in germinal centers and include somatic mutation and the selection of high-affinity cells (affinity maturation), additional isotype switching, memory B cell generation, and the generation of long-lived plasma cells.



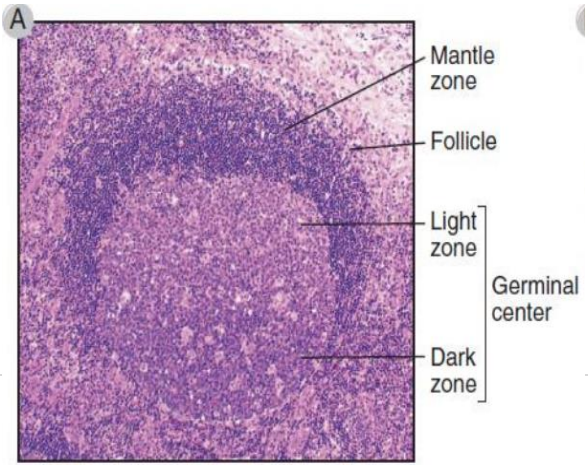
So, what happens within the germinal center?

1. affinity maturation (to have antibodies with better affinities), isotype switching (switching from IgM to IgG, IgE or IgA), generation of memory B cells, and long-lived plasma cell differentiation.

Histology of follicles>>>>

Notice dark and light zone

Within germinal center

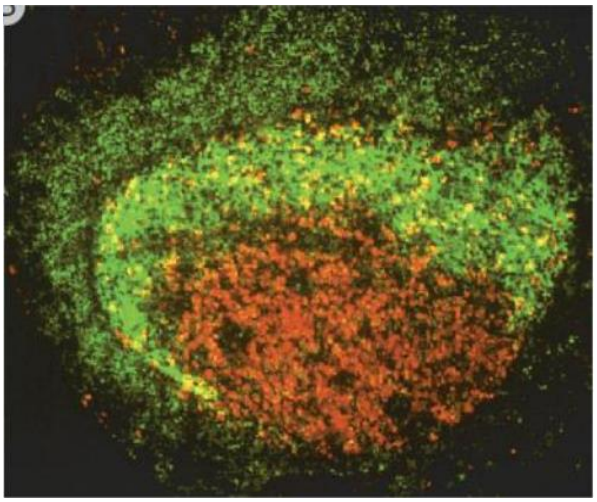


-Remember that primary follicle (before formation of germinal center) contains different naïve B-cells along with follicular dendritic cells.

-Again, germinal center has 3 main cells? **1.** Activated B-cells. **2.** Follicular T-helper cells. **3.** Follicular dendritic center.

-Germinal center is divided into: (refer to histology)

1. Dark zone: Part with lots of densely packed cells that are continuously proliferating.
2. Light zone: with lesser cells.



-Now, with immunofluorescence, we label cells with certain tags:

1. Green stain: labeled with Anti-CD23 antibody which is found on naïve B-cells with dimly green stain outside the germinal center.
2. Bright green (light zone): here there is higher density of CD23 found on Follicular dendritic cells.
3. Red (Dark zone): Detect cycling cells or mitotic active B-cells. Part of these B-cell migrate to light zone>>Yellow stain (due to mixing red with green stain), which may indicate B-cells that express CD23(Green)and highly mitotic(Red).

- We said that Follicular Dendritic cells (FDCs) participate in formation of germinal center by secreting chemokines to attract activated B-cells to follicle. They also help clean dead B-cells that undergo apoptosis in the follicle.

- FDCs are found only in lymphoid follicles and express complement receptors (CR1, CR2, and CR3) and Fc receptors, which helps them bind antigens that have deposited fragments of C3b, C3d and other complement proteins and in turn FCDs bind the antigen. FCDs also have Fc receptor since some antigens are bound to antibodies. **NOW**, after FCDs **catches** the antigen, they don't process it or present it with MCH-II, rather FCDs display antigen in its NATIVE conformation to B-cells (REMEMBER B-cells bind antigen in their native form, and FCDs can achieve that through its complement and Fc receptors).

- Now, the previous point occur within the germinal center in which B-cells are already activated! So WHY more activation occurs? Because within the germinal center we mentioned that 2 more reactions occur including: Affinity maturation and Isotype switching.

Summary:

As the figure indicate, there are mitotic active B-cells in dark zone, part of them migrate to light zone at which they either:

1. Interact with Antigen with native conformation expressed on FCDs through its complement and Fc receptors. (black arrow)
2. Through antigen-MHC-II complex expressed on B-cell, it interacts with TCR on Follicular T-helper cells, which starts secreting cytokines. (red arrow)
3. So, by these interactions' somatic maturation, affinity maturation and isotype switching occur. At the end, High affinity antibody-secreting plasma and memory cells are secreted.

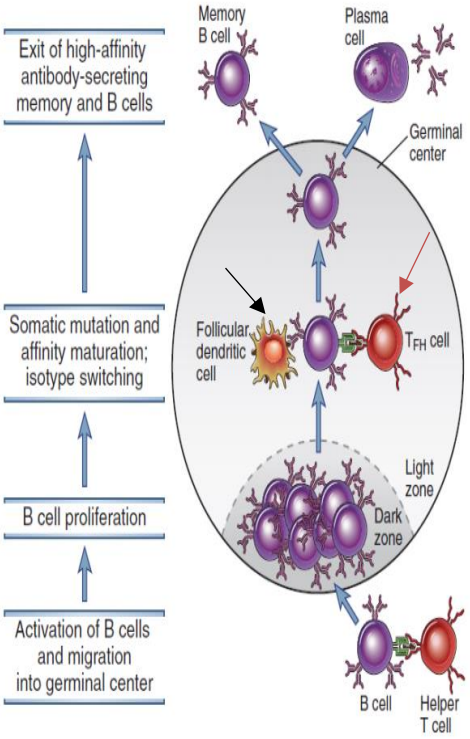


FIGURE 11–12 The germinal center reaction in a lymph node. B cells that have been activated by T helper cells at the edge of a primary follicle migrate into the follicle and proliferate, forming the dark zone of the germinal center. Germinal center B cells undergo extensive isotype switching. Somatic hypermutation of Ig V genes occur in these B cells, and they migrate into the light zone where they encounter follicular dendritic cells displaying antigen and T_{FH} cells. B cells with the highest affinity Ig receptors are selected to survive, and they differentiate into antibody-secreting or memory B cells. The antibody-secreting cells leave and reside in the bone marrow as long-lived plasma cells, and the memory B cells enter the recirculating pool.

- The following table indicate differences between Follicular focus (germinal center) and extrafollicular focus:

TABLE 11–1 Extrafollicular and Germinal Center B Cell Responses

Feature	Follicular/Germinal Center	Extrafollicular
Localization	Secondary follicles	Medullary cords of lymph nodes and at junctions between T cell zone and red pulp of spleen
CD40 signals	Required	Required
Specialized T cell help	T_{FH} cells in germinal center	Extrafollicular T helper cells
AID expression	Yes	Yes
Class switching	Yes	Yes
Somatic hypermutation	High rate	Low rate
Antibody affinity	High	Low
Terminally differentiated B cells	Long-lived plasma cells and memory cells	Short-lived plasma cells (life span of ~3 days)
Fate of plasma cells	Bone marrow or local MALT	Most die by apoptosis in secondary lymphoid tissues where they were produced
B cell transcription factors	Bcl-6	Blimp-1

AID, activation-induced cytidine deaminase; Bcl-6, B cell lymphoma 6; Blimp-1, B lymphocyte–induced maturation protein 1; IL-21R, interleukin-21 receptor; MALT, mucosa-associated lymphoid tissue; T_{FH}, follicular helper T cell.
Data from Vinusa CG, I Sanz, and MC Cook. Dysregulation of germinal centres in autoimmune disease. Nature Reviews Immunology 9:845-857, 2009.

Isotype switching:

-Switching immunoglobulins from IgM to IgG, IgE or IgA. It depends on cytokines that are secreted by TH-cell according to different types of microbes, to stimulate B-cell to class switch.

-HOW? In the germinal center, activated B-cell interacts with TH-cell, which will secret cytokines, DEPENDING on the microbe that initially activated B-cell.

-For example, in cases of viruses/some bacteria, TH1-cell will release IFN-**γ** (proved by experiment done in mice), leading to class switching to IgG, which make sense since this infection may last for a long time or may spread. So, IgG is superior in many aspects compared to IgM (coming from short lived plasma cells).

-**Note:** Remember that Polysaccharide antigens alone without help of T-cell (T-independent antigen) found on bacteria, can activate B-cells by cross-linking many receptors together and sending enough signals, stimulating B-cell to secret IgM to contain the infection quickly without the need of TH-cell/class switching/affinity maturation. But then we might need a prolonged immunity against infection by class switching from IgM to IgG through cytokines released by TH-cells. (Green arrow)

- IgM to IgE class switching: in case of helminthic infections (worm parasites) and hypersensitivity/allergic reactions (IgE have receptors on mast cells), through secretion of IL-4 by TH2-cell.

-Class switching isn't only determined by different microbes only, but also by different by different anatomical location, Specifically, B cells in mucosal tissues switch to IgA to produce mucosal immunity, for Ex. GI and respiratory tract. So, what happen is that we have lymphoid follicles near mucosal tissue that when activated become a germinal center, and TH-cells found within it, secret cytokines including: TGF-β, APRIL and BAFF that stimulate B-cell switching from IgM to IgA. IgA then goes through the epithelium into mucosal lumen.

- So, remember that isotype switching in B-cell occur within the light zone of germinal center by cytokines secreted by TH-cells.

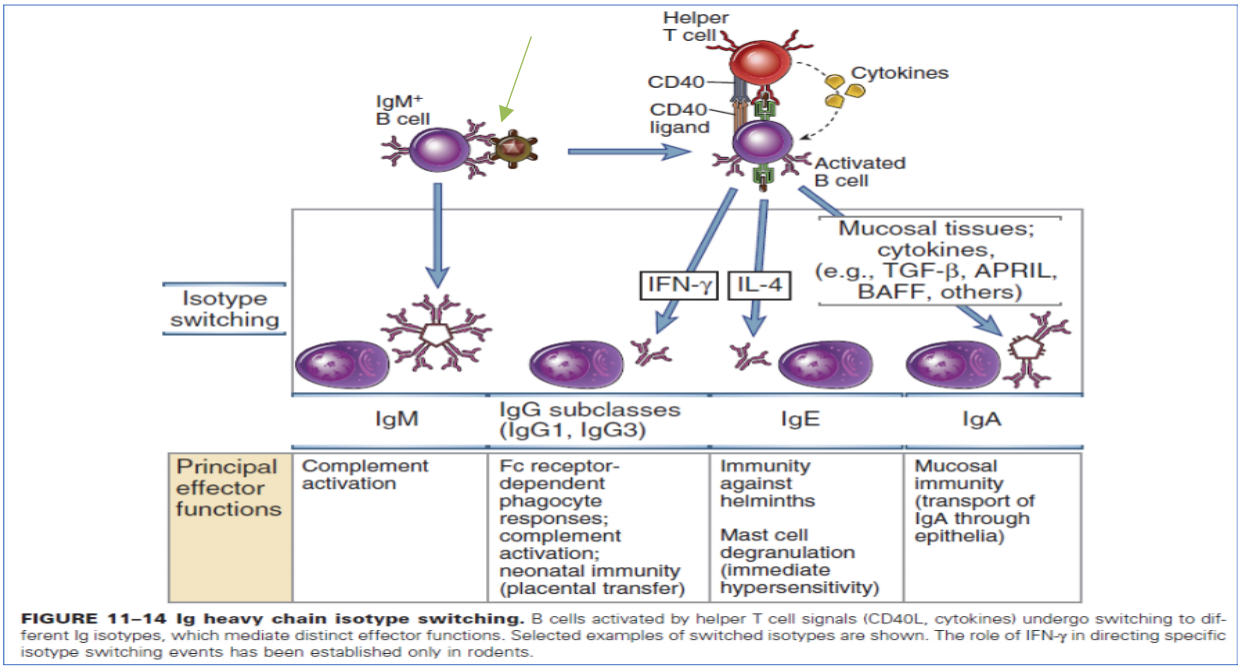


FIGURE 11–14 Ig heavy chain isotype switching. B cells activated by helper T cell signals (CD40L, cytokines) undergo switching to different Ig isotypes, which mediate distinct effector functions. Selected examples of switched isotypes are shown. The role of IFN-γ in directing specific isotype switching events has been established only in rodents.

Affinity maturation:

-It occurs in 2 steps: 1st somatic maturation of Ig genes followed by selection of B-cell with best somatic maturation.

-HOW?

1. Activated B-cells that were continuously proliferating in dark zone, mutate about 1000times more than normal cells in the body while they are replicating their DNA, especially in the variable region of the antibody, which will produce either high or low affinity antibodies. BUT remember we only need high affinity antibodies!
2. So here comes the next step, at which we need to select one with high affinity. So, B-cells with antibodies with different affinities when they migrate to light zone, there will be Follicular TH-cell that releases IL-21 cytokine which induce apoptosis of B-cells, unless these B-cells are rescued by a survival signal, which is binding of **HIGH AFFINITY** B-cell to antigens found on FCDs and also bind to Follicular TH-cell that release cytokines to keep them alive. So low affinity B-cells can't bind these cells>>apoptosis.

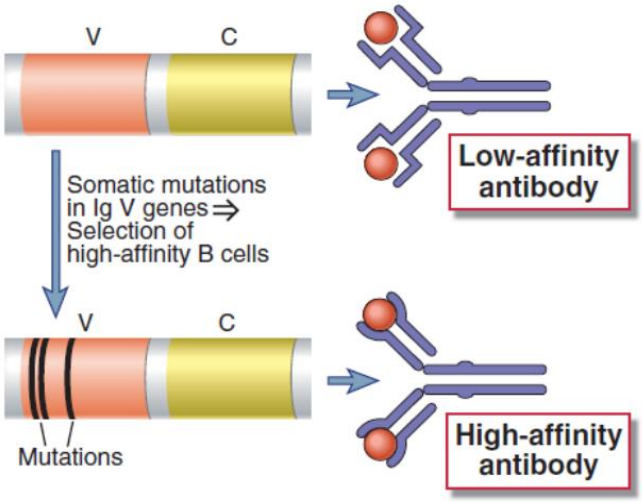


FIGURE 11–17 An overview of affinity maturation. Early in the immune response, low-affinity antibodies are produced. During the germinal center reaction, somatic mutation of Ig V genes and selection of mutated B cells with high-affinity antigen receptors result in the production of antibodies with high affinity for antigen.

-Note: Remember that mature naïve B-cells also receive survival signals in lymphoid follicle, that normally undergo apoptosis if it doesn't find its cognate antigen, unless they get a survival signal by binding to their antigen.

NOW, let's start with the 2nd part of our lecture, **Vaccination:**

-The aim of vaccination is to induce a protective immune response to the targeted pathogen without the risk of acquiring the disease and its potential complications by having already formed antibodies and memory B-cells.

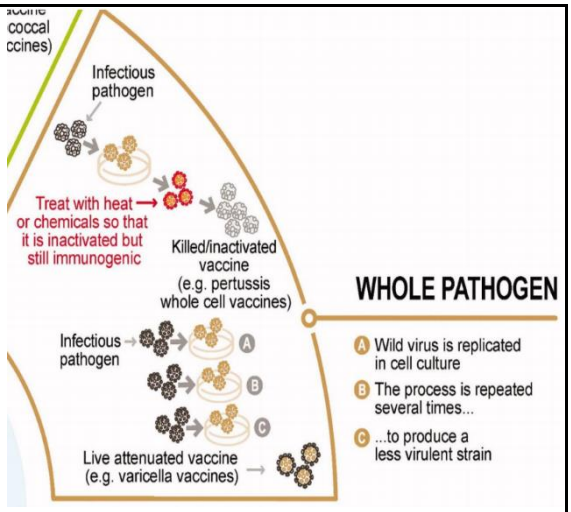
-HOW? By giving the patient the pathogen, so that the body develop antibodies against it OR give part of the pathogen(so that antibodies are formed against that part only), BUT remember you don't give the patient the pathogen itself since that would cause infection, you give a weakened form of the pathogen and this bring us to live-attenuated vaccines.

-**Live attenuated vaccines**: (mostly for viral infections)

- Live: means that its still living and can replicate | Attenuated: means its weakened.
- **How to attenuate it?**

1. The most common method to obtain live attenuated vaccines is to pass the virus through a series of in vitro cell cultures (e.g. in chick embryo cells). So according to following figure, you culture the pathogen within chicken embryo cells in culture A, and pathogens that survived are then moved from

culture A to culture B and so on…now with each passage (from A to B to…) the selected viruses become better at infecting and replicating in cell cultures BUT progressively lose their ability to infect and replicate in their original human host. So, these pathogens adapt best with cultures like chicken embryo cells BUT they are weak within human body.



2. Another method, you can culture it in a different temperature for Ex. 25 degrees, so some of these pathogen survive but others die, then you move the one that survived to another culture with 25degrees temp and so on…..so at the end this virus/pathogen adapt to environment with 25degrees so when its moved to the human body(37degrees body temp), it can't survive (weakened).

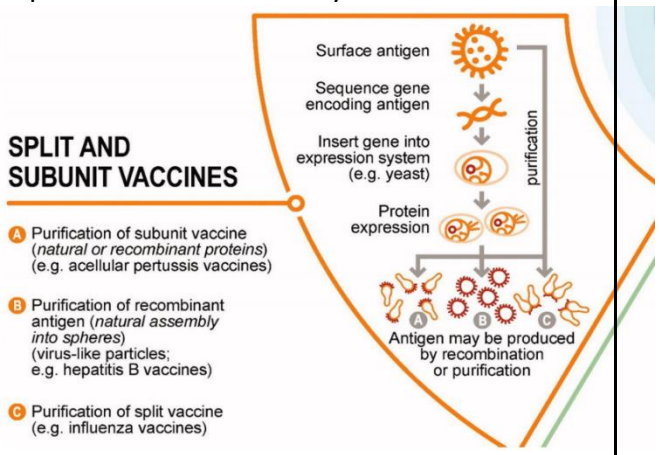
- So, the idea here that we adapt the pathogen to an environment in vitro culture producing a weakened form of it when injected in human body.
- REMEMBER THAT live attenuated vaccines are often contraindicated in immunocompromised individuals since they would cause infections.
- live attenuated vaccines often confer long term immunity after only one or two doses.
- SO, Live attenuated vaccines contain pathogens that have been weakened, altered or selected to be less virulent than their wild-type counterparts. In their altered form, they cannot cause the actual disease or only mimic the disease in a very mild way. They are generally produced from viruses rather than bacteria because viruses contain fewer genes and attenuation can be obtained and controlled more reliably.
- Classical examples of live attenuated vaccines produced by serial passage are those against measles, mumps, rubella and varicella, which are usually combined into trivalent or tetravalent vaccines. (Remember MMR live attenuated vaccine for the first 3viruses).
- IMPORTANT: The ONLY live attenuated bacterial vaccine currently in use is the bacillus Calmette-Guérin (BCG) vaccine given for tuberculosis which was developed almost a century ago, but still doesn't given good enough protection against TB. (1 in 5 people has TB, 25% of world's population is infected).
- Oral polio vaccine (OPV) is a live attenuated vaccine that was obtained through serial passages in non-human cells, OPV is easily administered through **oral drops, inexpensive, and effective** at inducing intestinal mucosal immunity (since polio first replication location is in the intestine and transmited veco-orally). After controlling this virus in USA, UK and most of Europe, they changed the vaccine from live attenuated to inactivated vaccine (killed form, so it can't cause any form of infection), since they completely

eradicated the virus, so why bring it back to community in a weakened form if its strong form is gone!

- However, in very rare cases (one case per million doses), OPV can mutate into a virulent form and induce very rare cases of vaccine-associated paralytic poliomyelitis, so there is a risk of infection but much lower risk than that without vaccination!

The 2nd type of vaccines is inactivated vaccine, in which pathogen is killed by treating it with heat/chemicals/radiation BUT still immunogenic.

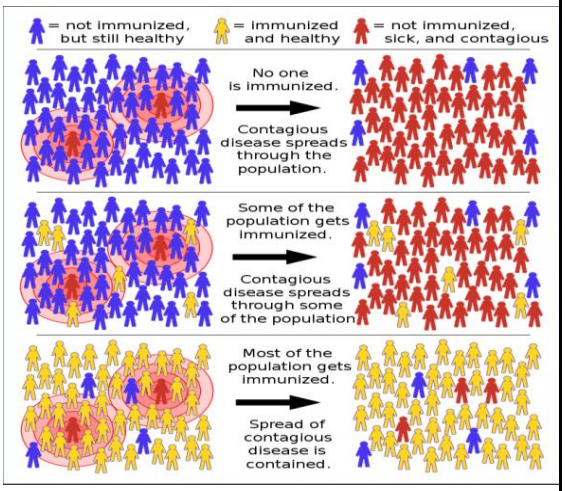
- Non-live vaccines do not contain any living or infectious particles, so they cannot cause disease and cannot reactivate. Therefore, they generally have a good safety profile, even in immunocompromised individuals.
- A drawback of these vaccines is that immunogenicity and duration of protection tend to be less than for live vaccines, so we give them in several doses or adjuvants to improve immunogenicity.
- Current examples of inactivated vaccines include the previously mentioned IPV (remember WE use OPV first!), whole-cell pertussis (bacteria that cause whooping cough), rabies and hepatitis A vaccines.
- You can also give part of non-live vaccine called **subunit vaccines**. These fragments can be proteins, polysaccharides, or parts of a virus that may form virus-like particles (VLPs).
- HOW to get subunit vaccines?
  1. You can get some of surface antigen and then purify it by certain molecular techniques.
  2. Or you can take the DNA from the pathogen and then put it in an expression system, which is a living system that can produce more of needed protein for e.g. yeast/E.Coli, used in protein expression.



- Examples of subunit vaccines,
  1. Acellular pertussis vaccines, so you purify antigenic proteins, and you would find 1 to 5 highly antigenic proteins. So, scientists scan all antigens within pathogen and choose one with highest antigenicity and that cause production of antibodies in the body.
  2. An example of recombinant protein vaccine is provided by the widely used hepatitis B vaccine in which the gene of the hepatitis B surface antigen (HBsAg) has been inserted into appropriate vectors for production in yeast. (Remember HBV surface antigen is important in diagnosis and IgG and IgM antibodies are produced against it during infection).

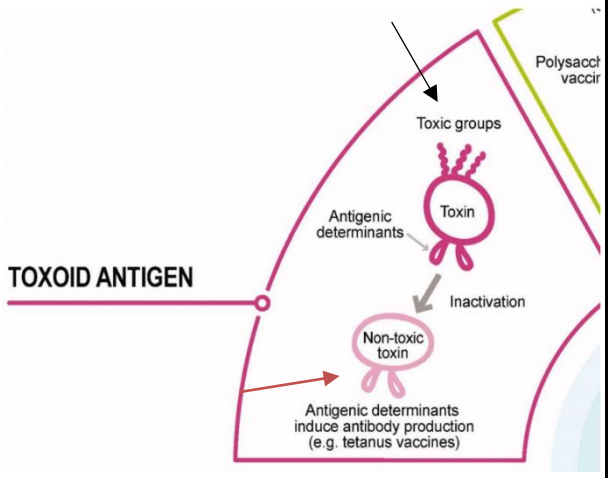
3. In some cases, you can combine 2 proteins together like in malaria vaccines (which is highly spread in Africa and south Asia), which isn't effective since the pathogen used plasmodium falciparum, doesn't produce enough immune response since most of its life is inside RBCs and antibodies can't reach it but it can limit the spread, so to solve this problem>>> the gene of a surface protein of the infectious form of Plasmodium falciparum is fused to the HBsAg gene, and the resulting recombinant fusion protein is expressed in yeast with free recombinant HBsAg which then become antigenic producing antibodies against it.

- **Toxoid vaccine** is a form of subunit vaccines, which is an inactivated toxin by heat and chemicals. REMEMBER, these vaccines are most important in bacteria that depend on toxins**. SUCH AS: 1.** Clostridium difficile or **2.** Corynebacterium diphtheriae (so you take its toxin, then inactivate it to form toxoid),**3.** Clostridium tetani (which produce tetanus toxin which works on neuromuscular junction causing continuous stimulation>>spastic paralysis). So, you produce a vaccine against that toxin by which you could have antibodies that bind and neutralize these toxins.
  - Toxoid have been used since their discovery in the 1920s.
  - IMPORTANT: Toxoids protect only against disease pathogenesis in vaccinated individuals BUT do not prevent infection or transmission. So, if its transmitted from vaccinated individual to non-vaccinated one, it will cause infection, so it doesn't provide Herd protection. (REMEMBER that previous vaccines, unlike toxoid, attack the pathogen itself NOT its toxins, preventing its replication, while Toxoid only effect toxin NOT the pathogen itself).
  - Note: Herd immunity is a form of indirect protection from infectious disease that occurs when a large percentage of a population has become immune to an infection, thereby providing a measure of protection for individuals who are not immune.

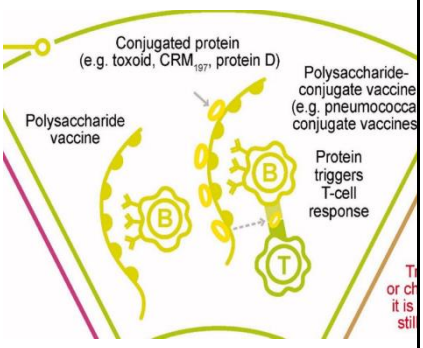


  - Refer to figure (to understand herd protection): we have 3 situations
    A. If you have a community that most of them aren't immunized(blue), if 1 or 2 are infected (Red), that get in contact with large number of non-immunized people, transmitting the disease to a large number of the community (contagious and easy spread!).

B. In 2nd case we have few people immunized but still when 1 or 2infected people come in contact with community, large numbers are still affected with few immunized people(yellow)
C. In 3rd case when most of the population is immunized, 1 or 2 infected individual when meet the community, level of spread of infection is very low.
D. So, the greater the proportion of individuals in a community who are immune, the smaller the probability that those who are not immune will come into contact with an infectious individual. Which is very important for immunocompromised patient who can't take live attenuated vaccines, so Herd protection is part of their immunity!



- Let's get back to toxoid vaccines, as the figure indicate, you take the toxin then inactivate it producing a non-toxic toxin which has the Antigenic determinant ONLY (Red arrow), since we removed the toxic groups (black arrow).
- Last type of non-lived vaccines is **<u>Polysaccharide and conjugate vaccines</u>**.
  - <u>Streptococcus pneumoniae, Hemophilus influenzae type b(Hib) and N. meningitidis</u> are three encapsulated bacteria, in which capsule helps them spread in blood and colonize the nasopharynx and other locations. So, these capsule must be dealt with, so they have taken the polysaccharide of the capsule and tried make a vaccine, which are poorly immunogenic, provide only short term protection because they will give a T-independent immune B-cell response, so it activate only B-cells without help of T-cell which will produce short-lived plasma cell that give IgM antibodies (Refer to page 5 **Note**). So, this vaccine only give protection for few days, so it was conjugated with proteins (Remember Hapten carrier effect in previous lecture), so Conjugation transforms the T-cell-independent response induced by polysaccharides <u>into</u>>>> a T-cell-dependent response that induces high-affinity antibodies and immune memory to have a long-term protection.



  - People who have undergone splenectomy, are highly susceptible to infections by the 3 encapsulated bacteria so they must be vaccinated.

Here ends Non-lived vaccines which include: whole pathogen, Subunit vaccines, Toxoid vaccines, Polysaccharide and conjugate vaccines.

- Adjuvants are substances that can enhance and modulate the immunogenicity of the antigen, but they aren't immunogenic on their own. It's not clearly known how it do that, but maybe due to activation of innate immune responses for e.g. aluminum salts (also known as alum) were the only adjuvant approved worldwide and they still remain the most widely used. adjuvants can broaden or extend responses and improve memory responses

- Adjuvants are usually not needed for live attenuated vaccines because these vaccines actively replicate and self-enhance the immune response.

-One of the drawbacks that face vaccinations:

1. in the case of HIV and Hepatitis C virus, that they have antigenic hypervariability, so their antigens keep changing even during the infection!
2. Some pathogens have multiple serotypes (e.g. dengue, S. pneumoniae)>>>S. Pneumonia have about 30-40types of capsules, so to make a vaccine against it, you collect the most common serotypes and collect them in a vaccine.
3. Pathogens that are always found intracellularly, can't be detected by antibodies or reduce their detection.

THE END (PLEASE REFER TO SLIDES)