IMMUNOLOGY_4TH SEM (ELISA) Swetangini Naik , Lect.Biotech.

Lecturer Notes:

What Is ELISA?

ELISA is a basic enzyme linked immunosorbent assay (also shortened as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies in the blood.

Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in certain infectious disorders.

ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwell plate.

Types of ELISA

ELISA tests can be classified into three types depending upon the principle of the structure of binding between antigen and antibodies, namely:

- **Indirect ELISA** – Antigen is coated to the microtiter well
- **Sandwich ELISA** – Antibody is coated on the microtiter well
- **Competitive ELISA** – Microtiter well which is antigen-coated is filled with antigen-antibody mixture.

Direct ELISA

- A buffered solution of the antigen to be tested for is added to each well (usually 96-well plates) of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of nonreacting protein, such as bovine serum albumin or casein, is added to each well in order to cover any plastic surface in the well which remains uncoated by the antigen.
- The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.
- The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more antibody is bound, the faster the color will develop. A major disadvantage of the direct ELISA is that the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or indirect ELISA provides a solution to this problem, by using a "capture" antibody specific for the test antigen to pull it out of the serum's molecular mixture.

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In

quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as the sample.

The use and meaning of the names "indirect ELISA" and "direct ELISA" differs in the literature and on web sites depending on the context of the experiment. When the presence of an antigen is analyzed, the name "direct ELISA" refers to an ELISA in which only a labelled primary antibody is used, and the term "indirect ELISA" refers to an ELISA in which the antigen is bound by the primary antibody which then is detected by a labeled secondary antibody. In the latter case a sandwich ELISA is clearly distinct from an indirect ELISA. When the "primary" antibody is of interest, e.g. in the case of immunization analyses, this antibody is directly detected by the secondary antibody and the term "indirect ELISA" applies to a setting with two antibodies.

Indirect ELISA

- Indirect ELISA detects the presence of antibody in a sample.
- The antigen is adhered to the wells of the microtitre plate.
- A sample containing the primary antibody is added to the wells which react with the coated antigen.
- The free primary antibodies are washed away and the antigen-antibody comples is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.
- Any free secondary antibody is washed away. A specific substrate for the enzyme is added which hydrolyzes to release a coloured product.
- The absorbance is measured by spectrophotometry.

Sandwich ELISA

- Sandwich ELISA helps to detect the presence of antigen in a sample.
- The antibody is coated on the microtitre well.
- An antigen containing sample is added to the well that reacts with the antibody present in the well.
- The well is washed and an enzyme-linked antibody specific for some other epitope on the antigen is added to react with it.
- The well is washed again to remove the free secondary antibodies and the substrate is added to the plate where it reacts with the enzyme to form coloured products.

Competitive ELISA

- Competitive ELISA helps to detect the concentration of antigen in a sample.
- The antibody is incubated in a solution with an antigen-containing sample.
- The antigen-antibody complex is added to the microtitre wells coated with antigen.
- The well is then washed to remove any unbound antibodies.
- The enzyme-conjugated secondary antibody specific for the isotype of primary antibody is added to determine the amount of primary antibody present in the well.
- The concentration is then determined by spectrophotometry.

Principle of ELISA

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

ELISA Procedure

ELISA is one of the easiest blood tests that can be carried out. It is rapid, quick and requires a blood sample of the patient. The entire procedure of ELISA is mentioned below.

- An antibody is attached to a polystyrene plate which is a solid surface and is attracted or has an affinity towards bacteria, other antibodies and hormones.
- A microtiter coated with antigen is filled with this antigen-antibody mixture after which unbound antibody is checked for and washed to remove.
- A second antibody specific to primary antibody is added which is usually a linked enzyme.
- Unbound enzyme-linked antibodies are removed by washing the plate.
- Finally, the substrate is added. The substrate is converted by the enzyme present, giving out a fluorescent signal.

HCG protein which indicates pregnancy is detected by ELISA. A combination of blood or urine sample and purified HCG linked to an enzyme is added to the system. If HCG is absent in the test sample, then only the linked enzyme binds to the solid surface.

The more the substance of interest is present, the more reaction takes place and less of linked enzyme binds to the solid surface. These reactions are indicated usually with a change in the colour of the solution.

Diseases That Can Be Diagnosed Using ELISA

ELISA can be used to detect some of these conditions:

- Ebola
- Pernicious anaemia
- AIDS
- Rotavirus
- Lyme disease
- Syphilis
- Toxoplasmosis
- Zika virus
- Carcinoma of the epithelial cells

Advantages of ELISA

Following are some of the advantages of ELISA technique:

- Results fetched from ELISA gives an accurate diagnosis of a particular disease since two antibodies are used.

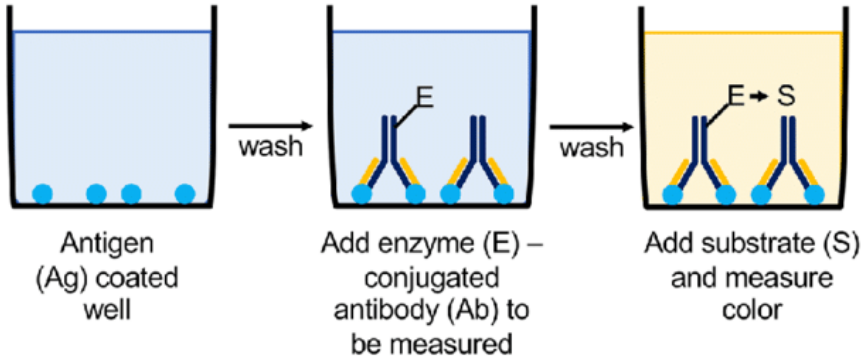
- Can be carried out for complex samples as antigen is not required to get purified to detect.
- It is highly responsive since direct and indirect analysis methods can be carried out
- It is a rapid test, yields results quickly
- Possible detection for ELISA ranges from the quantitative, semi-quantitative, standard curve, qualitative, calibration curve models etc.
- Easier to perform and uncomplicated process as compared to other assays which require the presence of radioactive materials.

Applications of ELISA

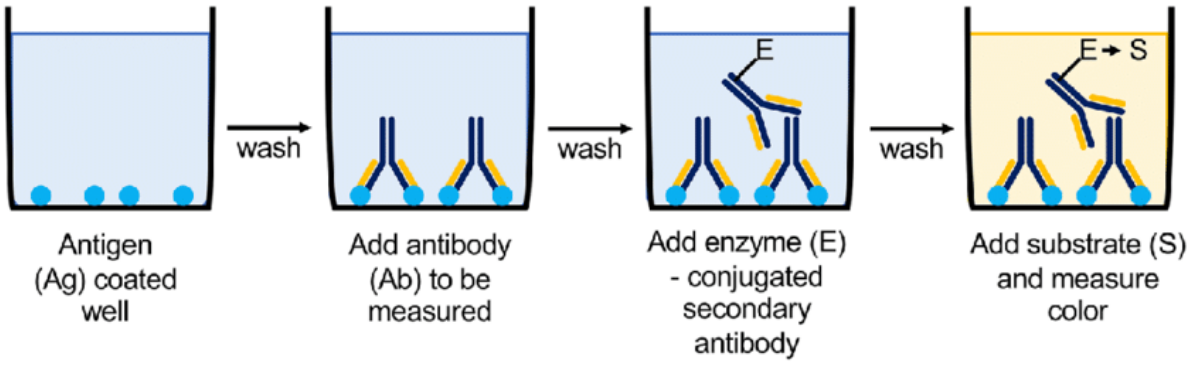
The applications of ELISA are discussed below:

1. The presence of antibodies and antigens in a sample can be evaluated.
2. It is used in the food industry to detect potential food allergens.
3. To determine the concentration of serum antibody in a virus test.
4. During disease outbreaks to track the spread of diseases.

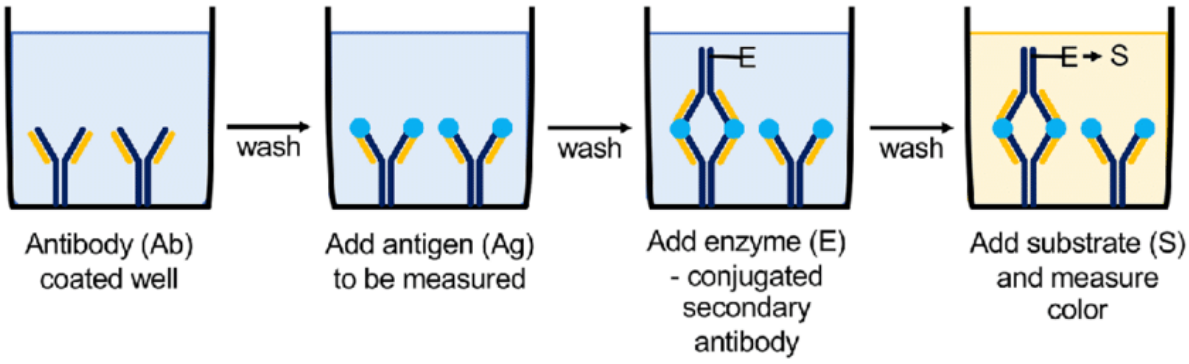
(a) Direct ELISA



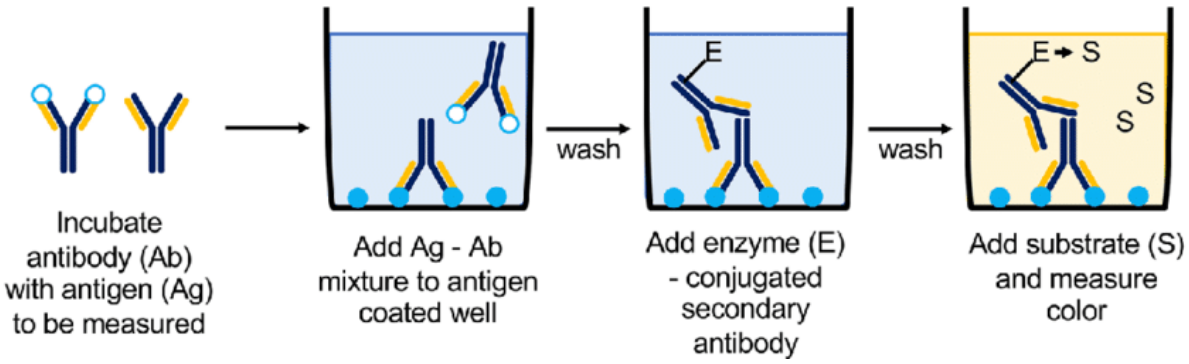
(b) Indirect ELISA



(c) Sandwich ELISA



(d) Competitive ELISA

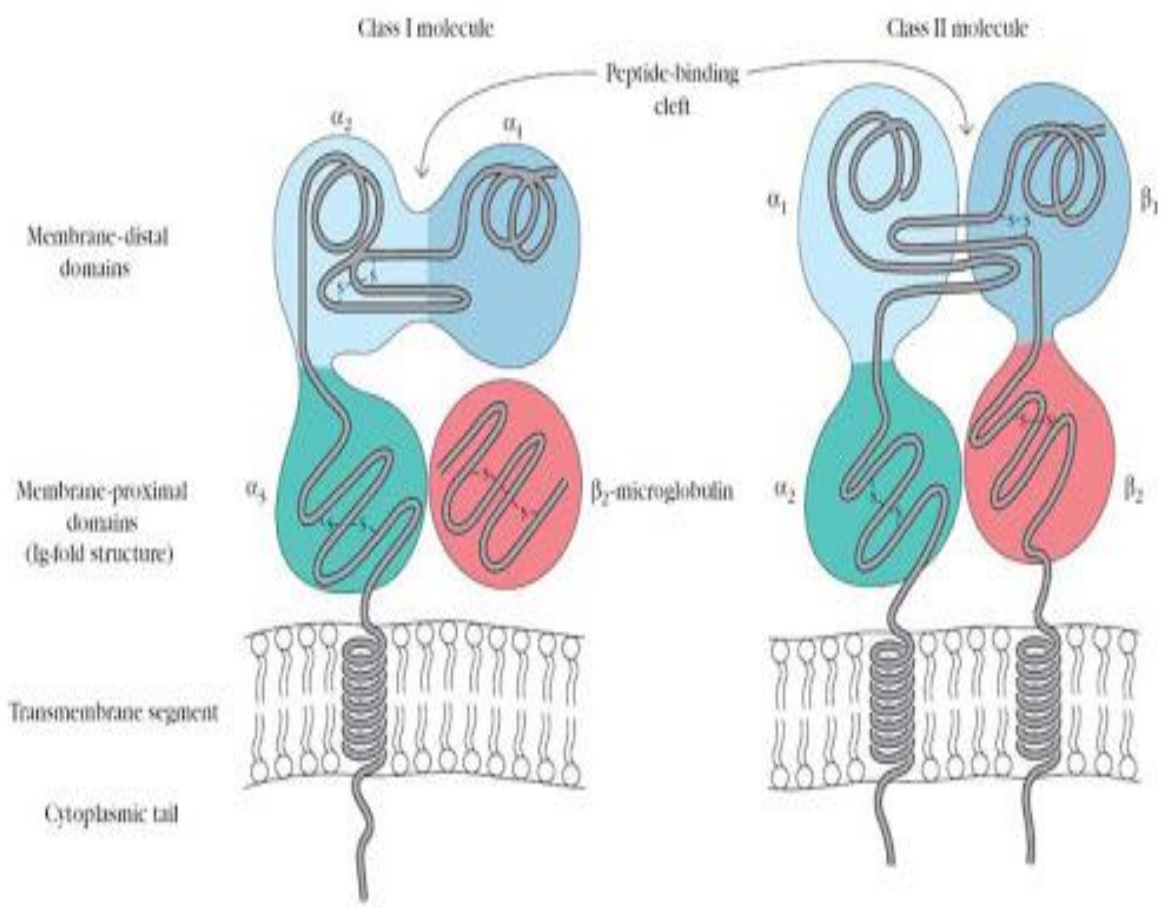


MHC class I molecules are one of two primary classes of major histocompatibility complex (MHC) molecules (the other being MHC class II) and are found on the cell surface of all nucleated cells in the bodies of vertebrates. They also occur on platelets, but not on red blood cells. Their function is to display peptide fragments of proteins from within the cell to cytotoxic T cells; this will trigger an immediate response from the immune system against a particular non-self antigen displayed with the help of an MHC class I protein. Because MHC class I molecules present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called *cytosolic* or *endogenous pathway*.

In humans, the HLAs corresponding to MHC class I are HLA-A, HLA-B, and HLA-C.

Structure

MHC class I molecules are heterodimers that consist of two polypeptide chains, α and β_2-microglobulin (B2M). The two chains are linked noncovalently via interaction of B2M and the α_3 domain. Only the α chain is polymorphic and encoded by a HLA gene, while the B2M subunit is not polymorphic and encoded by the Beta-2 microglobulin gene. The α_3 domain is plasma membrane-spanning and interacts with the CD8 co-receptor of T-cells. The α_3-CD8 interaction holds the MHC I molecule in place while the T cell receptor (TCR) on the surface of the cytotoxic T cell binds its α_1-α_2 heterodimer ligand, and checks the coupled peptide for antigenicity. The α_1 and α_2 domains fold to make up a groove for peptides to bind. MHC class I molecules bind peptides that are predominantly 8-10 amino acid in length (Parham 87), but the binding of longer peptides have also been reported.



(Structure of MHC I and MHC II

Function

Class I MHC molecules bind peptides generated mainly from degradation of cytosolic proteins by the proteasome. The MHC I:peptide complex is then inserted via endoplasmic reticulum into the external plasma membrane of the cell. The epitope peptide is bound on extracellular parts of the class I MHC molecule. Thus, the function of the class I MHC is to display intracellular proteins to cytotoxic T cells (CTLs). However, class I MHC can also present peptides generated from exogenous proteins, in a process known as cross-presentation.

A normal cell will display peptides from normal cellular protein turnover on its class I MHC, and CTLs will not be activated in response to them due to central and peripheral tolerance mechanisms. When a cell expresses foreign proteins, such as after viral infection, a fraction of the class I MHC will display these peptides on the cell surface. Consequently, CTLs specific for the MHC:peptide complex will recognize and kill presenting cells.

Alternatively, class I MHC itself can serve as an inhibitory ligand for natural killer cells (NKs). Reduction in the normal levels of surface class I MHC, a mechanism employed by some viruses[4] and certain tumors to evade CTL responses, activates NK cell killing.

MHC class II

MHC class II molecules are a class of major histocompatibility complex (MHC) molecules normally found only on professional antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. These cells are important in initiating immune responses.

The antigens presented by class II peptides are derived from extracellular proteins (not cytosolic as in MHC class I).

Loading of a MHC class II molecule occurs by phagocytosis; extracellular proteins are endocytosed, digested in lysosomes, and the resulting epitopic peptide fragments are loaded onto MHC class II molecules prior to their migration to the cell surface.

In humans, the MHC class II protein complex is encoded by the human leukocyte antigen gene complex (HLA). HLAs corresponding to MHC class II are HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR.

Mutations in the HLA gene complex can lead to bare lymphocyte syndrome (BLS), which is a type of MHC class II deficiency.

Structure[edit]

Like MHC class I molecules, class II molecules are also heterodimers, but in this case consist of two homogenous peptides, an α and β chain, both of which are encoded in the MHC.[1] The subdesignation α1, α2, etc. refers to separate domains within the HLA gene; each domain is usually encoded by a different exon within the gene, and some genes have further domains that encode leader sequences, transmembrane sequences, etc. These molecules have both extracellular regions as well as a transmembrane sequence and a cytoplasmic tail. The α1 and β1 regions of the chains come together to make a membrane-distal peptide-binding domain, while the α2 and β2 regions, the remaining extracellular parts of the chains, form a membrane-proximal immunoglobulin-like domain. The antigen binding groove, where the antigen or peptide binds, is made up of two α-helixes walls and β-sheet.[2]

Because the antigen-binding groove of MHC class II molecules is open at both ends while the corresponding groove on class I molecules is closed at each end, the antigens presented by MHC class II molecules are longer, generally between 15 and 24 amino acid residues long.

Expression

Expression

These molecules are constitutively expressed in professional, immune antigen-presenting cells, but may also be induced on other cells by interferon γ.[3] They are expressed on the epithelial cells in the thymus and on APCs in the periphery. MHC class II expression is closely regulated in APCs by CIITA, which is the MHC class II transactivator. CIITA is solely expressed on professional APCs however, non-professional APCs can also regulate CIITA activity and MHC II expression. As mentioned interferon-y (IFN-y) triggers the expression of CIITA and is also responsible for converting monocytes which are MHC class II negative cells into functional APCs that express MHC class II on their surfaces.

MHC class II is also expressed on group 3 innate lymphoid cells.

Importance

Having MHC class II molecules present proper peptides that are bound stably is essential for overall immune function.

[5] Because class II MHC is loaded with extracellular proteins, it is mainly concerned with presentation of extracellular pathogens (for example, bacteria that might be infecting a wound or the blood). Class II molecules interact mainly with immune cells, like the T helper cell (CD4+). The peptide presented regulates how T cells respond to an infection.[5] Stable peptide binding is essential to prevent detachment and degradation of a peptide, which could occur without secure attachment to the MHC molecule.[5] This would prevent T cell recognition of the antigen, T cell recruitment, and a proper immune response.[5] The triggered appropriate immune response may include localized inflammation and swelling due to recruitment of phagocytes or may lead to a full-force antibody immune response due to activation of B cells.

Roles:

- Development of humoral and cell-mediated immune response
- Antigen recognition by T cells *(most T cells recognize antigen only when it is combined with MHC molecule)*
- Determining whether transplanted tissue will be histocompatible or histoincompatible

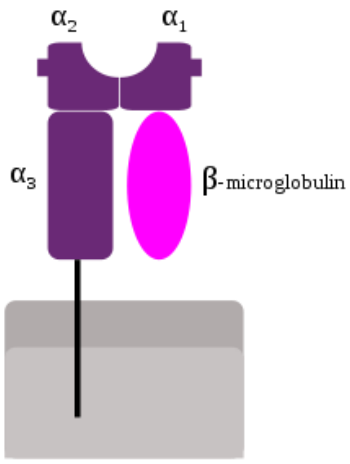
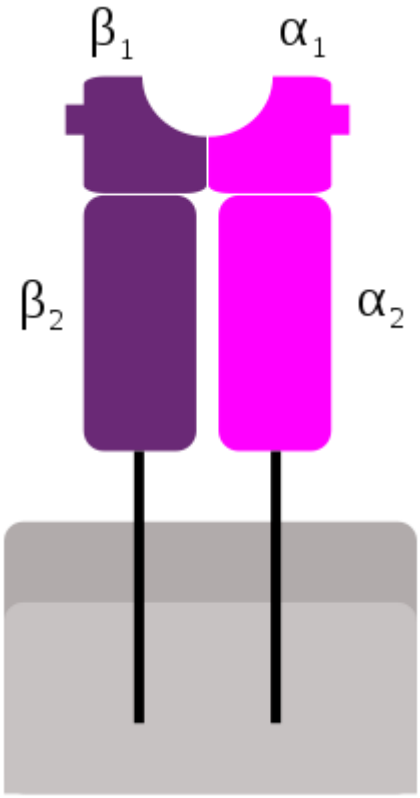
MHC Genes and Functions:

It is a collection of genes within a long stretch of DNA on chromosome 6, which codes for 3 classes of molecules

1. **Class I MHC** genes: encode glycoproteins expressed on the surface of nearly **all nucleated cells**; the major function of the class I gene product is **presentation** of peptide antigens to **TC cells.**
2. **Class II MHC genes** encode glycoproteins expressed primarily on **APCs**, where they present processed antigenic peptides to **TH cells**.
3. **Class III MHC genes** encode, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation (e.g. TNF, Heat Shock proteins).

Major difference between MHC Class I and MHC Class II (including their antigen processing and presentation pathway) is summarized in this table:

	MHC Class I	MHC Class II
Structure	MHC class I molecules consist of	MHC class II molecules consist of

	one membrane-spanning α chain (heavy chain) produced by MHC genes, and one β chain (light chain or β2-microglobulin) produced by the β2-microglobulin gene.  	two membrane-spanning chains, α and β, of similar size and both produced by MHC genes. 
Types of APCs	MHC I glycoproteins are present in all nucleated cells.	MHC II glycoproteins are only present on specialised antigen-presenting cells (APCs), including macrophages that engulf foreign particles such as bacteria, dendritic cells that present antigen to T cells, and B cells that produce antibodies.
Nature of Antigen Presentation	MHC class I glycoproteins present endogenous antigens that originate from the cytoplasm.	MHC II proteins present exogenous antigens that originate extracellularly from foreign bodies such as bacteria.
Size of peptide	MHC Class I present 8-10 amino acid peptides	MHC Class II presents 14-18 amino acid peptides.
Responsive T Cells	Present antigen to cytotoxic T cell lymphocytes (CD8+ T Cells);	Present antigen to helper T cell lymphocytes; (CD4+ T cells).
Co-receptor responsible	Binds with CD8 coreceptors molecules on cytotoxic T cells	Binds with CD4 co-receptors molecules on helper T cells
Sources of Protein Antigens	Cytosolic proteins (mostly synthesized in the cell, may enter cytosol from phagosomes)	Endosomal/lysosomal proteins (mostly internalized from extracellular environment)
Enzymes Responsible for peptide generation	Cytosolic proteasome	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of	Endoplasmic reticulum	Specialized vesicular compartment

MHC		
Molecules involved in transport of peptides and loading of MHC molecules	Chaperones, TAP in ER	Chaperones in ER; invariant chain in ER, Golgi and MHC Class II compartment/Class II vesicle; DM
End Result	Presentation of foreign-intracellular antigens or altered self-antigens; targets cell for destruction	Presentation of foreign extracellular antigens; induces antibody production, and attracts immune cells to area of infection

Antigen processing

Antigen processing, or the **cytosolic pathway**, is an immunological process that prepares antigens for presentation to special cells of the immune system called T lymphocytes. It is considered to be a stage of antigen presentation pathways. This process involves two distinct pathways for processing of antigens from an organism's own (self) proteins or intracellular pathogens (e.g. viruses), or from phagocytosed pathogens (e.g. bacteria); subsequent presentation of these antigens on class I or class II major histocompatibility complex (MHC) molecules is dependent on which pathway is used. Both MHC class I and II are required to bind antigen before they are stably expressed on a cell surface. MHC I antigen presentation typically (considering cross-presentation) involves the endogenous pathway of antigen processing, and MHC II antigen presentation involves the exogenous pathway of antigen processing. Cross-presentation involves parts of the exogenous and the endogenous pathways but ultimately involves the latter portion of the endogenous pathway (e.g. proteolysis of antigens for binding to MHC I molecules).

While the joint distinction between the two pathways is useful, there are instances where extracellular-derived peptides are presented in the context of MHC class I and cytosolic peptides are presented in the context of MHC class II (this often happens in dendritic cells).

The endogenous pathway

The endogenous pathway is used to present cellular peptide fragments on the cell surface on MHC class I molecules. If a virus had infected the cell, viral peptides would also be presented, allowing the immune system to recognize and kill the infected cell. Worn out proteins within the cell become ubiquitinated, marking them for proteasome degradation. Proteasomes break the protein up into peptides that include some around nine amino acids long (suitable for fitting within the peptide binding cleft of MHC class I molecules). Transporter associated with antigen processing (TAP), a protein that spans the membrane of the rough endoplasmic reticulum, transports the peptides into the lumen of the rough endoplasmic reticulum (ER). Also within the rough ER, a series of chaperone proteins,

including calnexin, calreticulin, ERp57, and Binding immunoglobulin protein (BiP) facilitates the proper folding of class I MHC and its association with β2 microglobulin. The partially folded MHC class I molecule then interacts with TAP via tapasin (the complete complex also contains calreticulin and Erp57 and, in mice, calnexin). Once the peptide is transported into the ER lumen it binds to the cleft of the awaiting MHC class I molecule, stabilizing the MHC and allowing it to be transported to the cell surface by the golgi apparatus.

The exogenous pathway

The exogenous pathway is utilized by specialized antigen-presenting cells to present peptides derived from proteins that the cell has endocytosed. The peptides are presented on MHC class II molecules. Proteins are endocytosed and degraded by acid-dependent proteases in endosomes; this process takes about an hour.[1]

The nascent MHC class II protein in the rough ER has its peptide-binding cleft blocked by Ii (the invariant chain; a trimer) to prevent it from binding cellular peptides or peptides from the endogenous pathway. The invariant chain also facilitates MHC class II's export from the ER in a vesicle. This fuses with a late endosome containing the endocytosed, degraded proteins. The invariant chain is then broken down in stages, leaving only a small fragment called "Class II-associated invariant chain peptide" (CLIP) which still blocks the peptide binding cleft. An MHC class II-like structure, HLA-DM, removes CLIP and replaces it with a peptide from the endosome. The stable MHC class-II is then presented on the cell surface.

Cross-presentation processing

In Cross-presentation, peptides derived from extracellular proteins are presented in the context of MHC class I. The cell starts off with the exogenous pathways but diverts the antigens (cytosolic diversion) to the endogenous pathway. This can allow the cell to skip the parts of the endogenous pathway that involve synthesis of antigens from the antigenic genes with cellular machinery upon infection, because the endogenous pathway can involve infection before being able to present antigens with MHC I, and cross-presentation saves them the effort needed for that and allows the professional antigen-presenting cells (dendritic cells) to process and present antigens without getting infected, which does not tend to happen to dendritic cells and is quite common scenario of antigen-processing using the endogenous pathway.[2] Not all antigen-presenting cells utilize cross-presentation.

B-cell activation with B-T cell interactions

Lymphocytes are one of the five kinds of white blood cells or leukocytes, circulating in the blood. Although mature lymphocytes all look pretty much alike, they are diverse in their functions. The most abundant lymphocytes are:

- B lymphocytes (often simply called B cells)
- T lymphocytes (likewise called T cells)

B cells are produced in the bone marrow. The precursors of T cells are also produced in the bone marrow but leave the bone marrow and mature in the thymus (which accounts for their designation). Each B cell and T cell is specific for a particular antigen, which simply means that each of these cells is able to bind to a particular molecular structure (such as an antigen). The specificity of binding resides in a specific receptor for antigen: the B-cell receptor (BCR) and the T-cell receptor (TCR) for B and T cells, respectively. Both BCRs and TCRs share these properties:

- They are integral membrane proteins.
- They are present in thousands of identical copies exposed at the cell surface.
- They are made before the cell ever encounters an antigen.
- They are encoded by genes assembled by the recombination of segments of DNA.

How antigen receptor diversity is generated

Each receptor has a unique binding site. This site binds to a portion of the antigen called an antigenic determinant or epitope. The binding, like that between an enzyme and its substrate, depends on complementarity of the surface of the receptor and the surface of the epitope and occurs mainly by non-covalent forces. Successful binding of the antigen receptor to the epitope, if accompanied by additional signals, results in:

1. Stimulation of the cell to leave G_0 and enter the cell cycle.
2. Repeated mitosis leads to the development of a clone of cells bearing the same antigen receptor; that is, a clone of cells of the identical specificity. BCRs and TCRs differ in:

- their structure
- the genes that encode them
- the type of epitope to which they bind

B cells

BCRs bind intact antigens (like diphtheria toxoid, the protein introduced in the diphtheria-tetanus-pertussis vaccine). These may be soluble molecules present in the extracellular fluid; or intact molecules that the B cell plucks from the surface of antigen-presenting cells like macrophages and dendritic cells. The bound antigen molecules are engulfed into the B cell by receptor-mediated endocytosis. The antigen is digested into peptide fragments by various protesomes and are then displayed at the cell surface attached along with a class II histocompatibility molecule. Helper T cells specific for this structure (i.e., with complementary TCRs) bind this B cell and secrete lymphokines that:

1. Stimulate the B cell to enter the cell cycle
2. The B cell undergoes repeated mitotic cell division, resulting in a clone of cells with identical BCRs;
3. The B cells switch from synthesizing their BCRs as integral membrane proteins to a soluble version;
4. The clonal cells differentiate into plasma cells that secrete these soluble BCRs, which we now call antibodies

T cells

There are two types of T cells that differ in their TCR:

1. alpha/beta (αβ) T cells: Their TCR is a heterodimer of an alpha chain with a beta chain. Each chain has a variable (V) region and a constant (C) region. The V regions each contain 3 hypervariable regions that make up the antigen-binding site.
2. gamma/delta (γδ) T cells: Their TCR is also a heterodimer of a gamma chain paired with a delta chain. They show characteristics of both innate immune response and acquired immune response; hence, regarded as the bridging between the two immune systems.

The discussion that follows now concerns alpha/beta T cells. The TCR (of αβ T-cells) binds a bimolecular complex displayed at the surface of some other cells called an antigen-presenting cell (APC). This complex consists of: a fragment of an antigen lying within the groove of a histocompatibility molecule. The complex has been compared to a "hot dog in a bun".

Vaccine Types

There are several different types of vaccines. Each type is designed to teach your immune system how to fight off certain kinds of germs — and the serious diseases they cause.

When scientists create vaccines, they consider:

- How your immune system responds to the germ
- Who needs to be vaccinated against the germ
- The best technology or approach to create the vaccine

Based on a number of these factors, scientists decide which type of vaccine they will make. There are 4 main types of vaccines:

- Live-attenuated vaccines
- Inactivated vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines

Live-attenuated vaccines

Live vaccines use a weakened (or attenuated) form of the germ that causes a disease.

Because these vaccines are so similar to the natural infection that they help prevent, they create a strong and long-lasting immune response. Just 1 or 2 doses of most live vaccines can give you a lifetime of protection against a germ and the disease it causes.

But live vaccines also have some limitations. For example:

- Because they contain a small amount of the weakened live virus, some people should talk to their health care provider before receiving them, such as people with weakened immune systems, long-term health problems, or people who’ve had an organ transplant.
- They need to be kept cool, so they don’t travel well. That means they can’t be used in countries with limited access to refrigerators.

Live vaccines are used to protect against:

- Measles, mumps, rubella (MMR combined vaccine)
- Rotavirus

- Smallpox
- Chickenpox
- Yellow fever

Inactivated vaccines

Inactivated vaccines use the killed version of the germ that causes a disease.

Inactivated vaccines usually don't provide immunity (protection) that's as strong as live vaccines. So you may need several doses over time (booster shots) in order to get ongoing immunity against diseases.

Inactivated vaccines are used to protect against:

- Hepatitis A
- Flu (shot only)
- Polio (shot only)
- Rabies

Subunit, recombinant, polysaccharide, and conjugate vaccines

Subunit, recombinant, polysaccharide, and conjugate vaccines use specific pieces of the germ — like its protein, sugar, or capsid (a casing around the germ).

Because these vaccines use only specific pieces of the germ, they give a very strong immune response that's targeted to key parts of the germ. They can also be used on almost everyone who needs them, including people with weakened immune systems and long-term health problems.

One limitation of these vaccines is that you may need booster shots to get ongoing protection against diseases.

These vaccines are used to protect against:

- Hib (*Haemophilus influenzae* type b) disease
- Hepatitis B
- HPV (Human papillomavirus)
- Whooping cough (part of the DTaP combined vaccine)
- Pneumococcal disease
- Meningococcal disease
- Shingles

Toxoid vaccines

Toxoid vaccines use a toxin (harmful product) made by the germ that causes a disease. They create immunity to the parts of the germ that cause a disease instead of the germ itself. That means the immune response is targeted to the toxin instead of the whole germ.

Like some other types of vaccines, you may need booster shots to get ongoing protection against diseases.

Toxoid vaccines are used to protect against:

- Diphtheria
- Tetanus

The future of vaccines

Did you know that scientists are still working to create new types of vaccines? Here are 2 exciting examples:

- **DNA vaccines** are easy and inexpensive to make — and they produce strong, long-term immunity.
- **Recombinant vector vaccines (platform-based vaccines)** act like a natural infection, so they're especially good at teaching the immune system how to fight germs.

Yellow Fever

Yellow fever is common in parts of Africa and South America. In fact, in Africa about 170,000 people get it every year. Yellow fever is not found in the United States — and thanks to the vaccine, travelers rarely get the disease.

The **yellow fever vaccine** is only recommended for people living in or traveling to places where yellow fever is a risk — or for people who work in labs studying the virus

U.S. Yellow Fever Vaccine Access

Sanofi Pasteur, the manufacturer of the only yellow fever vaccine (YF-Vax) licensed in the United States, has announced that YF-Vax will be unavailable from mid-2017 to mid-2019 because of delays in the production process.

Why is the yellow fever vaccine important?

Most people who get yellow fever will only get a mild form of the disease. But in some cases, people with yellow fever can develop serious complications — including organ failure or bleeding. Serious cases of yellow fever can be deadly.

If you’re planning to travel to parts of South America or Africa where yellow fever is common, or you work in a lab studying yellow fever, getting vaccinated can protect you

What is mumps?

Mumps is a disease caused by a virus. Symptoms of mumps include:

- Puffy cheeks and swollen jaw
- Fever
- Headache
- Muscle aches
- Feeling tired
- Not feeling hungry

Most people with mumps get better in a few weeks. But sometimes, it can cause serious complications, like:

- Inflammation of the lining of the brain and spinal cord
- Hearing loss
- Inflammation of the testicles in males who have reached puberty

Mumps spreads easily through the saliva (spit) of an infected person. It can spread when someone with mumps:

- Coughs, sneezes, or talks
- Shares cups or eating utensils (like a spoon) with other people
- Touches an object or surface that others might touch without washing their hands

- **Why is the mumps vaccine important?**
- Mumps is a contagious disease — it spreads easily from person to person. And it can lead to serious complications, like hearing loss.
- Although mumps is rare, infections can still happen in places where people are in close contact with each other — like schools, colleges, and camps.
- Getting vaccinated is the best way to prevent mumps. When enough people in a community get vaccinated for mumps, the entire community is less likely to get the disease. So when you and your family get vaccinated, you help keep yourselves *and* your community healthy.

There are 2 vaccines that can prevent mumps:

- The **MMR vaccine** protects children and adults from mumps, measles, and rubella
- The **MMRV vaccine** protects children from mumps, measles, rubella, and chickenpox

- **Why is the rubella vaccine important?**
- Rubella is a contagious disease caused by a virus. It can lead to serious complications, especially for unborn babies. If a pregnant woman gets rubella, she can lose her baby. Babies born to mothers who had rubella can have birth defects that last a lifetime.
- Rubella is still common in other countries. People can get the disease when they travel — and spread it to people who aren’t vaccinated when they come home.

- Getting vaccinated is the best way to prevent rubella. And when enough people get vaccinated against rubella, the entire community is less likely to get it. So when you and your family get vaccinated, you help keep yourselves *and* your community healthy.

What is rubella?

Rubella is a disease caused by a virus. Sometimes, rubella doesn't cause any symptoms. When it does cause symptoms, they may include:

- Mild fever
- Headache
- Mild pink eye (redness or swelling of the eye)
- Swollen glands
- Feeling uncomfortable
- Cough
- Runny nose

Most people with rubella get better in a few weeks. But sometimes, it can cause serious complications, like:

- Arthritis (joint pain and swelling)
- Brain infections
- Bleeding problems

Rubella is very dangerous for unborn babies. If a woman gets rubella during pregnancy, she can lose her baby — either earlier in the pregnancy (miscarriage) or later in the pregnancy (stillbirth). Babies born to mothers with rubella can also have serious health problems that last for life. For example:

- Heart problems
- Hearing or eyesight loss
- Learning disabilities
- Liver or spleen damage

Rubella spreads through the air — like when someone who has it coughs or

What is rotavirus?

Rotavirus is caused by a virus, and it mostly affects babies and young children. Symptoms of rotavirus include:

- Severe diarrhea
- Throwing up
- Dehydration
- Fever
- Stomach pain

- Changes in behavior

Rotavirus spreads when a person comes in contact with the poop of someone who has rotavirus and then touches their own mouth. For example, rotavirus can spread when a child with rotavirus doesn't wash their hands properly after going to the bathroom and then touches food or other objects.

Why is the rotavirus vaccine important?

Rotavirus is a contagious disease — it spreads easily from child to child. Rotavirus can cause diarrhea (watery poop), which can lead to dehydration (not having enough water in the body). Children who get severe cases of rotavirus may need to be hospitalized.

The rotavirus vaccine protects 9 out of 10 children from getting severe illness caused by rotavirus.

The rotavirus vaccine is the best way to protect your child from rotavirus.

What is measles?

Measles is a disease caused by a virus. Symptoms of measles include:

- Fever
- Rash
- Cough
- Runny nose
- Mild pink eye (redness or swelling of the eyes)

Sometimes, measles can lead to:

- Ear infections
- Diarrhea (watery poop)
- Pneumonia (lung infection)
- Inflammation of the brain

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- **Why is the measles vaccine important?**
- Measles is one of the most contagious diseases there is. If 1 person has it, 9 out of 10 people close to that person who aren't immune (protected) will also get measles. And it can be dangerous — serious cases of measles can lead to brain damage and even death.
- In recent years, measles outbreaks have increased in the United States and around the world in places like Europe, Africa, and South America. Outbreaks typically happen in areas where groups of people don't get vaccinated. Since measles is still common in other countries, people can get the disease when they travel — and spread it to people who aren't vaccinated when they come home.
- Getting vaccinated is the best way to prevent measles. And when enough people get vaccinated against measles, the entire community is less likely to get it. So when you and your family get vaccinated, you help keep yourselves *and* your community healthy.

Measles spreads through the air — like when someone who has it coughs or sneezes. The virus can live for up to 2 hours in the air.

The smallpox vaccine protects people from smallpox by helping their bodies develop immunity to smallpox. The vaccine is made from a virus called **vaccinia**, which is a poxvirus similar to smallpox, but less harmful. The smallpox vaccine contains live vaccinia virus, not a killed or weakened virus like many other vaccines. For that reason, people who are vaccinated must take precautions when caring for the place on their arm where they were vaccinated, so they can prevent the vaccinia virus from spreading.

The vaccine does not contain the smallpox virus and cannot give you smallpox.

For most people with healthy immune systems, live virus vaccines are effective and safe. Sometimes a person getting a live virus vaccine experiences mild symptoms such as rash, fever, and head and body aches. In certain groups of people, complications from the vaccinia virus can be severe. The Smallpox Vaccine Safety page has more information about who is more likely to experience these side effects.

Other live virus vaccines currently used include measles, mumps, rubella, and chickenpox.

Smallpox vaccination can protect you from smallpox for about 3 to 5 years. After that time, its ability to protect you decreases. If you need long-term protection, you may need to get a booster vaccination. Find out who should get smallpox vaccine.

Historically, the vaccine has been effective in preventing smallpox infection in 95% of those vaccinated. In addition, the vaccine was proven to prevent or substantially lessen infection when given within a few days after a person was exposed to the variola virus.

Routine smallpox vaccination among the American public stopped in 1972 after the disease was eradicated in the United States.

What is chickenpox?

Chickenpox is caused by a virus. Symptoms of chickenpox include:

- A red, itchy skin rash with blisters
- Fever
- Feeling tired
- Not feeling hungry
- Headache

Chickenpox usually spreads when a person touches chickenpox or shingles blisters — or if they breathe in the virus. You can breathe in the virus after someone with chickenpox or shingles scratches their blisters, which releases the virus into the air.

It's also possible to get chickenpox from breathing in tiny droplets from people who have it that get into the air after they breathe or talk. Learn more about chickenpox

Why is the chickenpox vaccine important?

Chickenpox is very contagious — it spreads easily from person to person. And while it's usually mild, it can cause serious complications like pneumonia (lung infection). Certain people — like infants, people with weakened immune systems, and pregnant women — are at increased risk for complications.

The chickenpox virus can also cause shingles later in life. Shingles is a disease that causes a painful skin rash and can affect the nervous system. Children who get the chickenpox vaccine may have a lower risk of developing shingles later on — and those who do get shingles often have a milder case than someone who has had chickenpox.

Getting vaccinated is the best way to prevent chickenpox. And when enough people get vaccinated against chickenpox, the entire community is less likely to get it. So when you and your family get vaccinated, you help keep yourselves *and* your community healthy

What is hepatitis A?

Hepatitis A is a liver disease caused by a virus. Some people with hepatitis A don't have any symptoms. Other people do develop symptoms, including:

- Fever
- Feeling tired
- Upset stomach and throwing up
- Not feeling hungry
- Dark pee or clay-colored poop
- Pain in the joints and stomach
- Jaundice (yellow skin or eyes)

Symptoms usually last less than 2 months — but they can last as long as 6 months.

Hepatitis A usually spreads when someone eats or drinks something that has come in contact with the poop of someone with the hepatitis A virus. For example, hepatitis A can spread when someone who has it doesn't wash their hands properly after using the bathroom and then touches food.

Hepatitis A can also spread from person to person through sexual contact.

Why is the hepatitis A vaccine important?

Because of the vaccine, rates of hepatitis A in the United States are the lowest they've been in 40 years. But hepatitis A is still common in other countries, so it's possible for people to get the disease when they travel.

Most people who get hepatitis A only get a mild form of the disease. But in some cases, hepatitis A can lead to serious liver problems — and even death.

Getting vaccinated is the best way to prevent hepatitis A.

What is polio?

Polio is caused by a virus. Most people who get polio don't have any symptoms. When people do get symptoms, they may include:

- Sore throat
- Fever
- Upset stomach
- Headache
- Stomach pain

Sometimes polio can affect the brain, and lead to serious — and permanent — complications like:

- Paresthesia (feeling pins and needles)
- Inflammation of the lining of the brain and spinal cord
- Paralysis (not being able to move)

Polio usually spreads when someone gets certain body fluids or poop from a person with polio on their hands and then touches their own mouth. Polio spreads when:

- Someone who has polio coughs or sneezes
- Someone who has polio doesn't wash their hands properly after going to the bathroom and then touches food or objects

Why is the polio vaccine important?

Polio is a very contagious disease — it spreads easily from person to person. Most people who get polio don't have any serious problems. But in some cases, polio can be very dangerous and lead to permanent disabilities — and even death.

Even though it's rare in the United States, polio still exists in a few countries in Asia and Africa. So it's possible for people to get polio when they travel — and spread it to people who aren't vaccinated when they come home.

When you and your family get vaccinated, you're doing your part to make sure that polio doesn't become a problem in the United States again.

What is rabies?

Rabies is caused by a virus that can be passed to humans through the bite of a rabid animal (an animal who has it). People in the United States are most likely to get rabies from wild animals, especially bats. Animals like raccoons, skunks, and foxes may also spread rabies. It's also possible to get rabies from pets, like dogs and cats, that haven't been vaccinated. In countries where rabies is still common, people often get it through the bite of a rabid dog.

Rabies doesn't generally spread from person to person — though very rarely, it could spread from one person to another during an organ transplant.

Early symptoms of rabies include:

- Weakness
- Fever
- Headache

As the disease gets worse, rabies can cause:

- Trouble sleeping
- Feeling confused
- Anxiety and agitation (feeling nervous, worried, or upset)
- Seizures (sudden, unusal movements or behavior)
- Hallucinations (seeing things that aren't there)

Once a person shows symptoms of rabies, they almost always die.

Why is the rabies vaccine important?

Though it's rare in the United States, people who get rabies almost always die. In the United States people are most likely to get rabies from wild animals. Rabies is more common in other countries.

If you've been bitten by an animal that could have rabies, or are at risk of coming in contact with rabies, it's very important to get the vaccine.