INTRODUCTION TO MEDICAL

IMMUNOLOGY

SLIDE SHEET

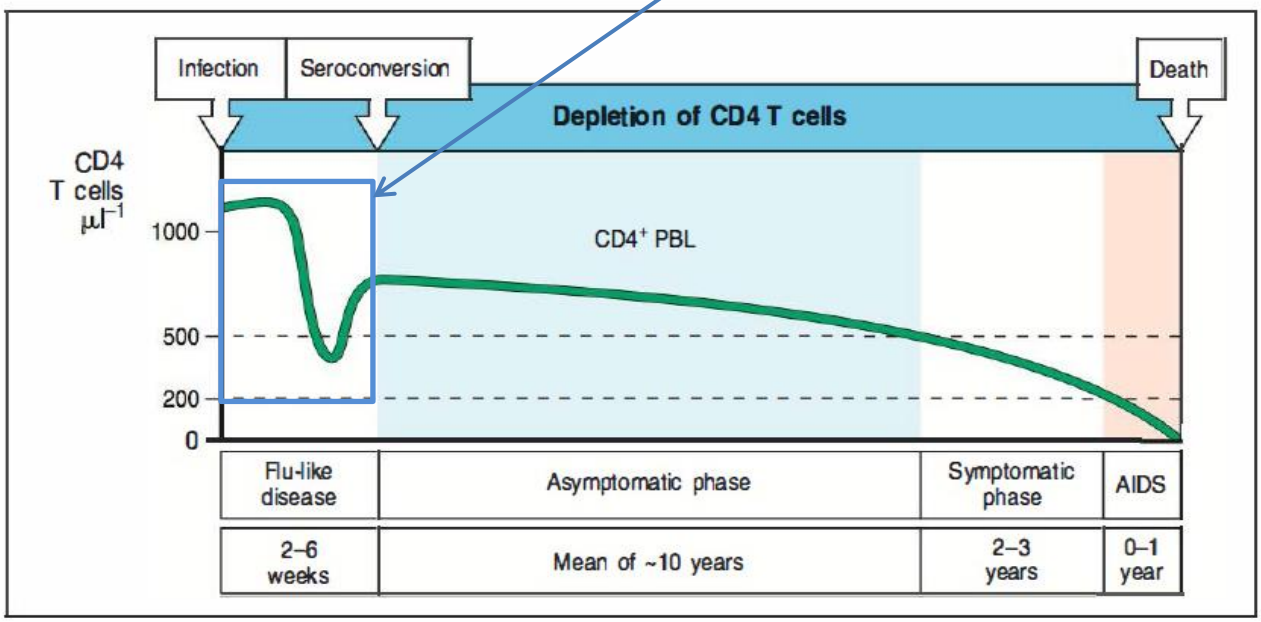
NUMBER	2 (Lec #23 + #24)
DONE BY	Mariam Hassouneh
CORRECTION	Dr.Malik Sallam
DOCTOR	Dr.Malik Sallam

Salam Everyone ♥ … It's been a day filled with hard work for sure! So may Allah bless what's also left and be always along this way beside you. Wishing this would be a simple sheet, and hopefully you'd enjoy it :D

Today we'll be completing with secondary immunodeficiency by talking about HIV/AIDS as the most common cause of secondary immunodeficiency related to infections, and then we'll start a new topic which is primary immunodeficiency.

** (Please refer to case studies book as it's the Doctor's reference for studying the case.) These are some comments after the case was presented by **Enas Ajarma** and **Lina Lawama**.

1. Most important determinant for progression was thought to be CD4 cells count, but recently, the viral load (virus concentration in the blood as determined by quantitative PCR) at the viral set point has been shown to be a better determinant for progression to AIDS.
2. Important to remember that Gut Associated Lymphoid Tissue (GALT) is the most affected Sec.Lymph.Organ in Acute HIV phase.



3. Target cells (cells susceptible to be infected) are any cell that has CD4 "along with " CCR5 or CXCR4 as Co-Receptors.

Deficiency in CCR5 (like CCR5 Δ32 that produce a non-functional CCR5 protein) doesn't prevent the individual from being infected due to the ability of the virus to use OTHER Co-receptors to enter the cells which is due to "**Redundancy of immune system**".

Further explanation?? Sure!

CCR5 is a protein that functions normally in the body as chemokine (**chemo**tactic cyto**kine**) receptor, and since we have a lot of receptors in immune system that act as chemokine receptors (families of CXC, CC, CX3C, XC), if a single type of a family (which is here CCR5) is defected, **No** clinical manifestations **due to this defect ALONE** will appear " in HIV infected or in normal non HIV infected people" because others will compensate- which is good. At the same time, what's bad is if HIV viruses encountered this defected Co receptor, they can use other Co-receptors (e.g. X4 viruses -if HIV- can use CXCR4 (also called X4) as the co-receptor, hence, will be capable of cellular entry and infection). This is what we call Redundancy " وفرة " of the immune system; meaning that we have many molecules doing THE SAME FUNCTION.

Quick RECAP: Immune system redundancy applies perfectly to chemokines as well as their receptors since there are **plenty of them exerting the same function** (advantage to the immune system), and so **No clinical manifestations** should be seen in case certain type got defected. BUT, this redundancy isn't seen for example when I have deficient Abs, as we'll notice that B cells will be affected and as a consequence an **IMMUNODEFICIENCY** will occur leaving the person susceptible mostly to encapsulated bacteria (Bacteria having a bacterial capsule made of polysaccharide; e.g. *Streptococcus pneumoniae, Neisseria, H. influenza*) in Respiratory tract.

4. In the case study, even though the husband was HIV infected and married since 2 years, his wife was HIV –ve, HOW could this be??!
   Risk of transmission of HIV through different transmission roots (heterosexual intercourse, needle injection and drug abusing, vertical transmission, ..) varies much.
   a. The possibility of infecting upon **one** heterosexual transmission is less than 1%.
   What are the risk factors that increase this percentage?? Viral load -and how high it is- which will reflect how many viruses we'll have in genital tract that'll be site of sexual contact, so not every sexual contact will result in HIV transmission. However, precautions should be taken into account as no one would want to take a chance no matter how low the probability of HIV acquisition is!

b. Upon needle stick injury (health care workers are exposed to get infected when they take blood samples from patients that may or may not know they're HIV infected), the estimated probability is 0.3%.

* Note: Such percentages are obtained from systematic reviews, by collecting all research papers that discussed prevalence of the disease by a certain transmission route.

hypothetical example: paper from UJ hospital that indicated 10 needle stick injuries from a person known to be HIV positive + paper form King Abdullah the 2nd Hospital also indicating 10 injuries, and so on, until we reach **1000** injuries, to see that only **3** people who got injured had a **sero-conversion** (meaning that they developed immunity and **Abs** against HIV **which were detected in their serum).** By doing calculations: 3/1000 ×100% = **0.3%**

** Note that: 0.3% and other percentages aren't exactly the actual exact percentages but they're an approximation reflecting the risk of transmission of a certain infection through a certain mode of transmission.

- For your info: Infectiousness of hepatitis C through needle stick injury = 3%

Infectiousness of hepatitis B through needle stick injury = 30%

For sake of Exam: Such numbers won't come as they are but they'll come between confidence intervals (ex. 0.1% – 0.5% instead of just 0.3).

Dr.Malik noted the following: I do not like this kind of questions that depend on memorizing numbers, as the numbers are mere estimation of the actual figures. However, it is important to know a rough estimation of these figures. MCQ example:

Bad MCQ in my opinion:

-The number of people living with HIV infection globally by the end of 2016 is:

a. 36.7 million.

b. 31.7 million.

c. 40.7 million.

d. 41.7 million.

e. 30.7 million.

Good MCQ in my opinion:

-The number of people living with HIV infection globally by the end of 2016 is:

a. 31-43 million.

b. 1-5 million.

c. 100-120 million.

d. 200-200 million.

e. less than a million.

5. Modes of HIV transmission:

a. Blood "body fluids" as in transfusion of blood or its products.
b. Sexual transmission; whether hetero or homosexual.
c. Injection drug use.
d. Vertical transmission (mother to child) which may occur during intrauterine life, delivery (intrapartum), or postpartum through breastfeeding.

Highest risk of transmission of HIV- infection cases comes from receiving non- screened blood (95%),

Then Vertical transmission from untreated mother (25%),

Then Homosexual transmission and injection drug use,

Then Heterosexual transmission.

20.05min – 24.25min (Lec 23) + 00.00min – 11.05min (Lec 24)

Primary immunodeficiency

Today we'll start talking about Primary immunodeficiency "PID", as we previously discussed ins and outs of secondary immunodeficiency.

We mentioned previously that when we're suspicious that a certain person is immunodeficient, **recurrent** and **opportunistic** infections were our key words, but we should also consider a third factor, which is the **SEVERITY of common infections** in these patients.

* Common cold might pass us by without causing significant manifestations that could threaten our lives or disturb our daily routine that much, but in these immunodeficient patients, emergency rooms might be waiting!

- Primary immunodeficiency clinical signs:

1. Positive family history (inherited genetic defect).

2. Infections in multiple anatomic locations.

3. Increased frequency and severity of infections with age.

4. Recurrent serious infections with common pathogens.

5. Serious Infections with unusual organisms: We previously mentioned some of these organisms like *Candida* and *Pneumocystis jirovecii* (the most famous in HIV patients).

- Suppose that depending on a patient's signs and symptoms, we became very suspicious that he might be immunodeficient, **what's the work up that should be done to diagnose the patient** and to affirm his immunodeficient state??

Note: **Work up means what laboratory tests, radiologic investigations among other investigations should we do when a patient comes with a chief compliant (Chief رئيسية; compliant شكوى; chief compliant شكوى رئيسية) in order **to reach the correct Diagnosis**.

1) Clinical history: **The most important part of reaching the correct diagnosis** (more important than physical examination, radiology, lab tests); It's when you take patient's **chief complaint** (which is the answer to the question "**What brought you to the hospital**?"), ask about past and present history, write his apparent signs and note symptoms which he'll tell us about.
   It's Important to ask questions that'll enable you to exclude diseases and keep others to reach your differential diagnosis (Dx).

Ex.1 The patient has a cough.

You set in your mind a Dx list containing upper respiratory tract infections, lower respiratory tract infection, allergies, and so on; then by asking questions, you start limiting your choices until reaching a possibly true Dx.

Ex.2 A patient may come to ER with chest pain among other symptoms like nausea, heartburn, difficulty in breathing.

The differential diagnosis is the list of conditions which would be manifested with the same chief complaint (chest pain in this example). The list for chest pain includes acute coronary syndrome (MI and angina), pneumonia, gastroesophageal reflux disease, etc.

To reach the final diagnosis we should take a careful clinical history and do a physical exam, ECG, CXR (chest X-ray), lab tests (e.g. troponin level etc.)

** 70% of the real Dx can be reached by taking clinical history, the rest 30% are distributed on physical examination and lab tests.

2) Physical examination: mentioned previously; it's when you examine the patient's body to look for disease signs.

3) Lab tests:

a) **The first and most simple one is CBC** (complete blood count). What matters us in the case of immunodeficient patient is the count of WBCs, which should be LOW!
But even though, this isn't always true, WHY?? Remember that WBCs include neutrophils, basophils, eosinophils, monocytes, and lymphocytes. Neutrophils are elevated during bacterial infections (Neutrophilia) as a compensation in case of deficiency in lymphocytes, so this will reflect an overall elevated WBCs count, that's why we'll need **differential WBCs count** to see which subset of cells are low in number.

b) **Quantitative Immunoglobulins**. Used to test **humoral immunity** and so B cells activity; as there are many PIDs that affect B cells (Ex. Hyper IgM syndrome), so the best way to test B cell activity is by testing presence of its products which are Igs.
Blood concentration of IgG is the highest, followed by IgA and IgM, then IgD, lastly IgE.
****** Tested Igs in routine lab tests are IgG, IgM, and IgA.

c) **Reviewing previous culture results**. We here look for previous microbiologic cultures to see if there were any evidences for DOCUMENTED increase in frequency of infections or infection with unusual microorganisms.

d) **Titers for administered vaccines**. WHY? Here we also test humoral response by measuring serum concentration for certain Abs formed against vaccines' antigens.

Ex. Finding Abs against surface antigen of hepatitis B means there was a response against the antigen when hepatitis B vaccine was administered, and so the result will be +ve. If not found, titers will be –ve, meaning there is a problem in response to vaccine, meaning there is a problem in Ab production, and so problem in humoral immunity.
** Note that: Absence of Ab response to certain vaccines might be related to other factors so immune deficiency is not always the underlying condition.

e) **Lymphocyte enumeration using FCM.** Enumeration means counting; what we do here is counting Lymphocytes using Flow Cytometry (FCM).

** Generally, Cells in a fluid or suspension are tested using 2 ways:

1. Direct microscopic examination by placing the sample on a slide and count under microscope.

2. **By FCM** (Figure 1 illustrates the mechanism).

Following notes related to FCM are optional and will not be included in the exam.

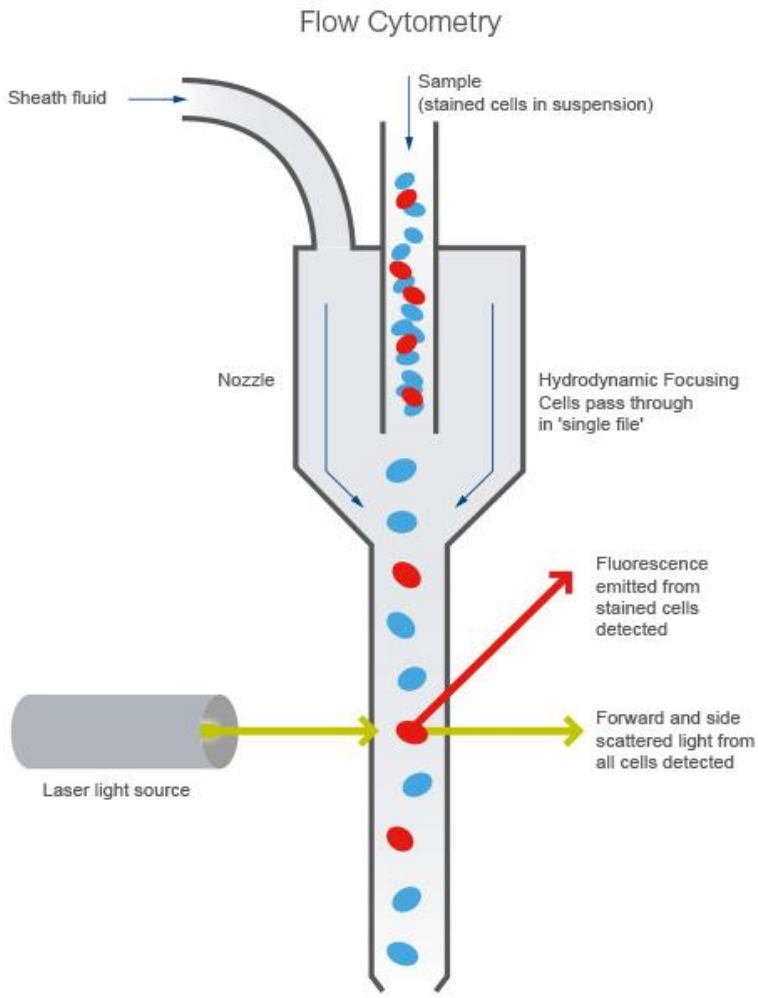


Figure 1 : Flow cytometry

We pass the suspension through part of the FCM, then by measuring light absorption and deflection characteristics of the suspension cells -or any particles passed- we'll be able to tell [1)]**cells sizes**, [2)]**cells granularity**, and thus indirectly the **complexity of these cells** (like having multi-lobed nucleus).

We can also know [3)] **cells immunophenotype** (Figure 1 illustrates the mechanism); It refers to "what antigens do I have on cells surface, intracellular, or intranuclear?"

Ex. Different cell Surface markers termed “Clusters of differentiation; CD“ are found on lymphocytes (Pic 3 illustrates CD markers). B cells have Pan surface markers (**Pan** means **all** types of cells) known as CD19 and CD20, T cells have a Pan marker known as CD3, while CD16 and CD56 are surface markers for Natural killers.

This immunophenotyping is done by labeling a certain CD marker Ab (Ex. We’re looking for CD3) with florescent molecule, we incubate these Abs with our cells, if these cells have CD3 marker which we’re looking for on them, the Florescent Abs will bind to CD3, if it didn’t exist “Cells incubated were B cells”, Abs won’t bind. After washing out any Ab that didn’t bind during incubation, we pass the cells through part of the FCM that’ll release a laser light with short wave-length, and the Abs that’ll absorb this short wave-length light energy and emit the light in a longer wave-length will be detected by sensors inside the FCM and so the **existence** plus the **amount** of **wanted type of cells** will be shown (Figure 4 illustrates an FCM immunophenotype).

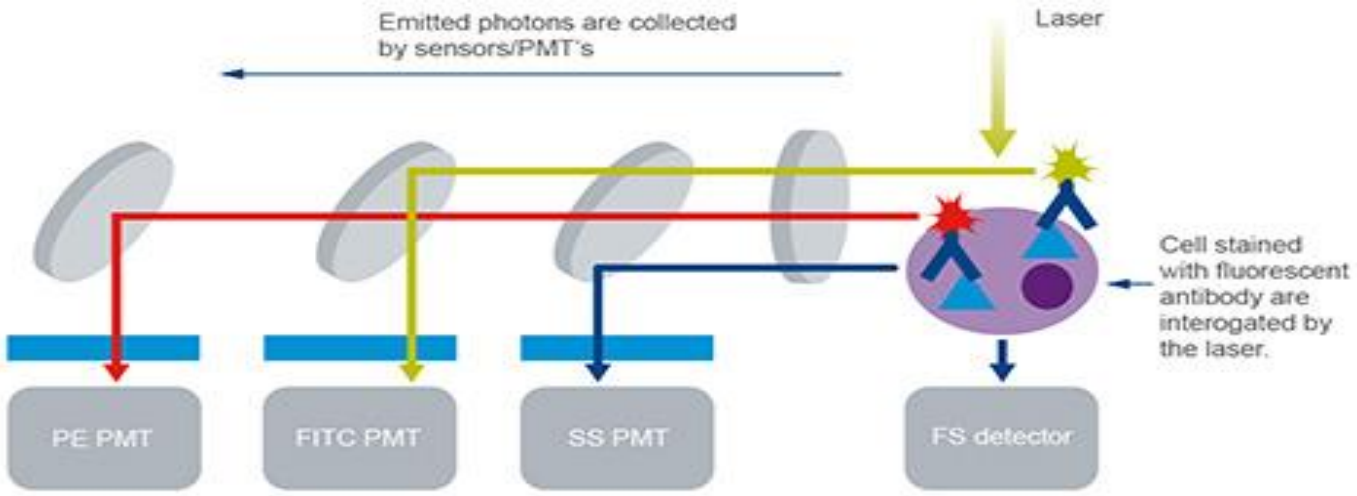


Figure 2: Detecting CD markers

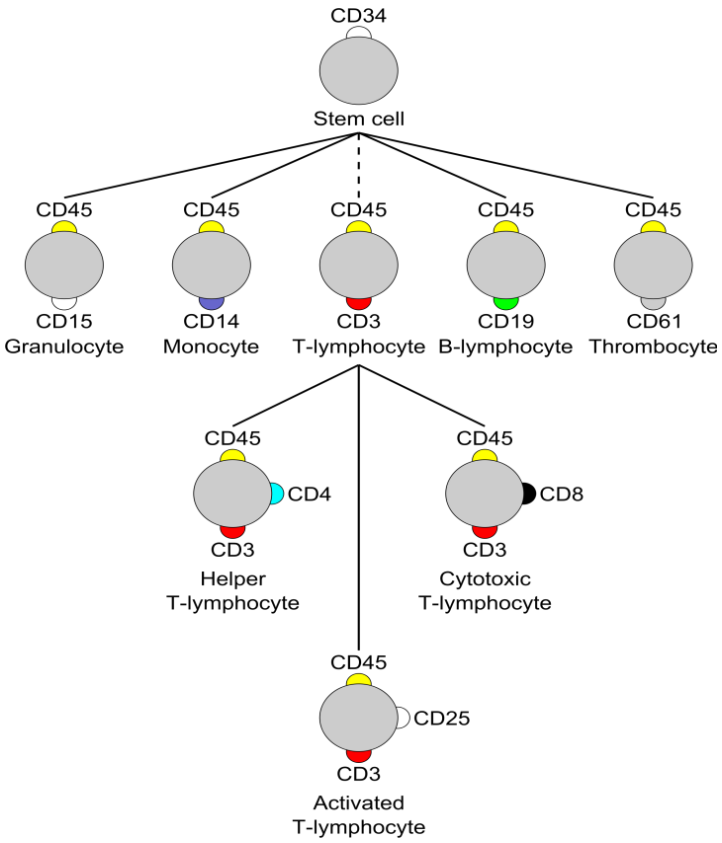


Figure 3: Clusters of differentiation

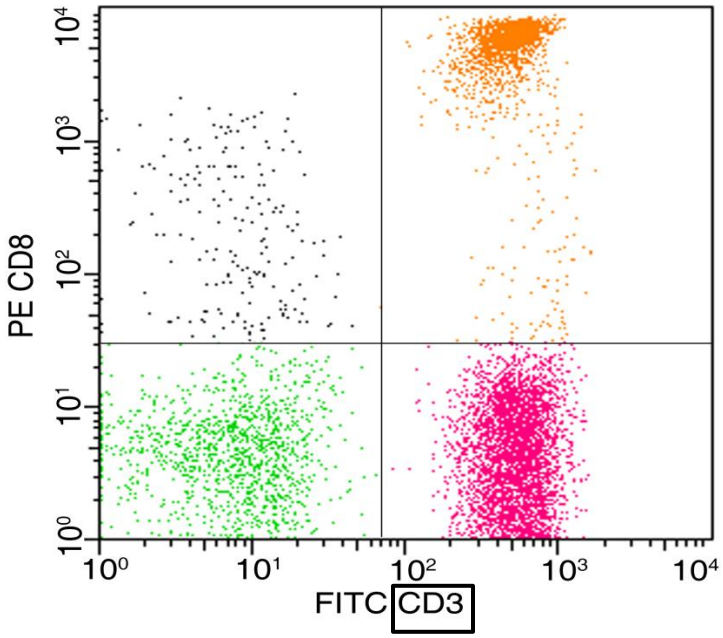


Figure 4: FCM immunophenotyping

11.05min - 25.45min

f) **Skin testing**. An indirect measure to cellular immunity (T cells); like tuberculin test.

*** Note: This part will be included in the exam.**

Handout 10: "In this test we inject the purified tuberculin under the skin, and check the area in a few days. If the patient has active TB or have been infected with it in the past, his immune system will include memory, Th1-type cells that were made in response to the infection. After the injection, dendritic cells present beneath the skin take up the protein and present tuberculin peptides to memory cells – and they are reactivated. These Th cells secrete IFN-γ and TNF – Th1-type cytokines that activate resident tissue macrophages near the site of injection, and help recruit neutrophils and additional macrophages to the area. The result is a local inflammatory reaction with redness and swelling: the signal that TB test is positive. Of course, the reasons we have to wait several days for the test to "develop" are that memory helper T cells must be reactivated, proliferate, and produce those all-important cytokines that orchestrate the inflammatory reaction.

On the other hand, if the patient **has never been exposed to the tuberculosis mycobacterium**, he will have no memory helper T cells to reactivate. Without the cytokines supplied by activated Th cells, there will be no inflammatory reaction to the tuberculin protein, and the skin test will be **negative**."

g) **CH50, C3 & C4.**

Measuring the complement components concentration, most commonly C3 and C4, are used to determine whether deficiencies or abnormalities in the complement system are causing, or contributing to, a person's disease or condition.

- ✓ CH50 is measuring the 50% Hemolytic Complement Activity (CH50); it's used to look at the integrity of the entire complement pathway from C1 to C9; any deficiency in any complement component will be reflected in abnormal CH50 result.

  - How is this test done??

  The CH50 tests the functional capability of serum complement components of the **classical** pathway to lyse sheep red blood cells (SRBCs). A sample of the patient's blood is brought to be tested (Because complement proteins are very fragile and should be preserved, blood sample tube should be put **in pack filled with ice** and transported **immediately** to the laboratory. You will see that when you start your clinical years.), we place sheep RBCs sensitized by Rabbit Abs (these Abs are anti-sheep red blood cell antibody), we add then patient's serum sample, **If Hemolysis to these SRBCs was observed**, then we can tell that classical pathway is intact, but if not (-ve result), we'll have to suspect the deficiency in at least single type of complement components.
- ✓ C3 and C4 are ordered because mostly they have the highest concentration among other components.

** For your info: Most common complement component to be deficient is C2.

h) **Phagocyte studies**. Used to test the activity of Neutrophils and macrophages; the most famous test to be ordered is **Nitro blue tetrazolium test (NBT)** which is one of the tests we discussed when we talked about **Chronic granulomatous disease** in the first case study .

Handout 4: "Nitro blue tetrazolium dye (NBT) is a pale yellow dye used to detect if there is H2O2. The dye is mixed with blood from the patient and if H2O2 is present it will be reduced and its color becomes purple". So in CGD since there's NADPH oxidase deficiency in phagocytes, no ROSs will be formed so cells color will stay the same.

i) **Enzyme studies**. Some enzymes when deficient it'll cause Primary immunodeficiency, like Activation-induced Cytidine deaminase deficiency (AID deficiency) which we took as 2nd case study, and it was the cause of Hyper IgM syndrome.

As we already said, PIDs are very rare; almost >150 types of PIDs were recognized until now, most of them result from Monogenic gene deficiency "a single gene is only defected".

(Figure 5 illustrates the distribution of PIDs by type).

MOST COMMON component of immune system to be a PID target is **humoral immunity**; genetic defects targeting B cells activity and causing a primary immunodeficiency are very common; and the most common PID targeting B cells is **Selective IgA deficiency** (**which is also the most common of overall PIDs**; refer to sheet 1, page 5 to check).

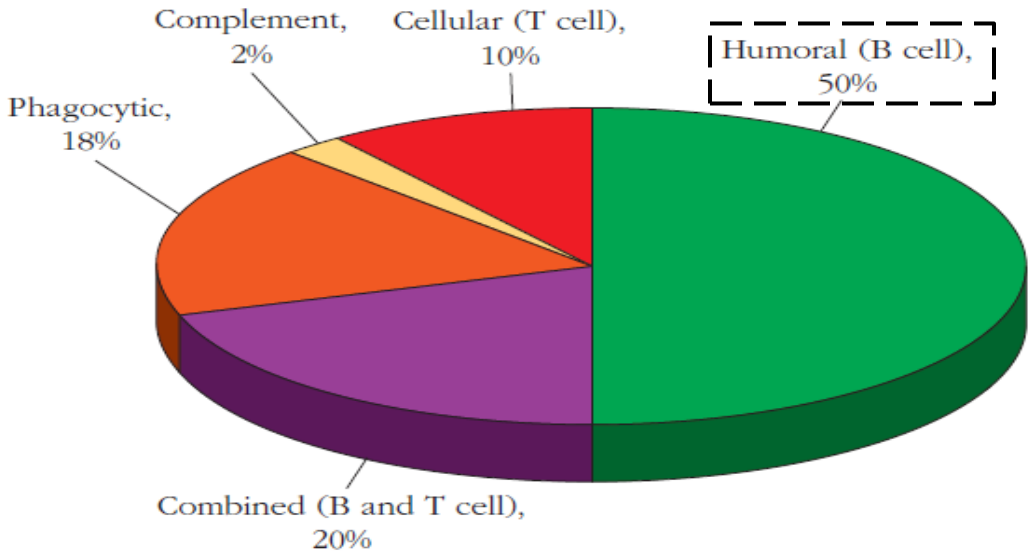


Figure 5: Distribution of Primary immunodeficiencies by type.

** PID can involve either innate processes (Phagocytosis, complement, or other defects), or the adaptive immune response (humoral, Cellular, or both like SCID). Of these categories, **ADAPTIVE immune disruptions are the most common,** with Ab defects making up the largest portion of these.

** Severity of the primary immune deficiency itself ranges from mild, to moderate, to severe, to even fatal!

This depends on level of immune deficiency; If PID is in STEM cells, it would be severe to fatal, but if it was manifested in more mature cells, it would be milder in course.

Ex. In most Selective Immunoglobulin deficiencies, like in Selective IgA deficiency, the patient might reach adulthood (his 20s or 30s) without knowing that he/she has this disorder.

25.45min - 29.55min

Next lecture: We'll be discussing Disease-specific primary immune deficiency. What is required from you to know is:

1. Names of PID disorders
2. Which component of immune system is deficient
3. Underlying genetic cause
4. What kind of infections will take place?

VERY Important Note from Dr.Malik to Section #3: "The exam will be from what was given to you in the lectures and this is my full responsibility, to make sure that you are given the same lecture material as the two other sections."

Finally you're DONE!!

Congratulations *.*, I'm sure there's a bunch of sheets on the table waiting for you, so what a great hero you are!

If you found anything that should be corrected or enhanced please don't hesitate to inform me with!

Wishing you always blessed days, and remember that no matter how tired you felt, or no matter what things you went through, you will reach that day at the end when people draw that wide smile on your face and say to you: " شكراً دكتور، أنقذت حياتي " :D