Children with recurrent infections

objectives:

- Definition and prevalence of primary immunodeficiency (PID) diseases.
- History taking and physical examination of children with suspected PID.
- Examples of common and prototypic PIDs (e.g.: SCID, XLA, CGD, DiGeorge syndrome, WAS, AT, LAD, complement deficiency).
- Diagnostic approach to PIDs.
- Therapeutic approach to PIDs.

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Notes

Important

Book

Overview

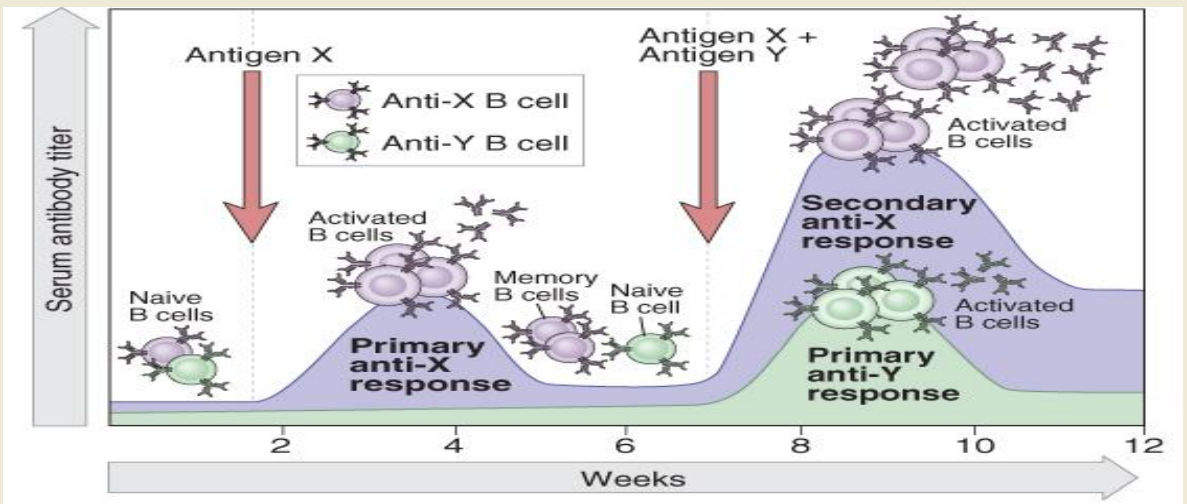
Host Immune Defense Mechanisms

Nonspecific (innate)		Specific (adaptive)
Barriers: - Skin - Enzyme secretions (mucous, tears, saliva) - Mucociliary clearance, peristalsis		Humoral (Which is a function of B-lymphocytes) By this we mean mainly the production of antibodies.
Cytokines	Phagocytes	Cellular (Mainly a function of T-lymphocytes)
Complement System - Neutrophils - Macrophages		

Innate vs Adaptive

- Quick early response. - 1st, 2nd & 3rd exposures are similar (rapidity & strength of response). - Not specific (in general) it may differentiate between groups of organisms(gram-negative,gram-positive bacteria, fungi & viruses) but cannot differentiate between different antigens therefore cannot differentiate exactly between different microbes.	- Take some time. - Memory is a function of adaptive immune system (can remember previous exposures) - Can differentiate (even parts of antigen) because of inherent ability to recognize more antigens in the environment more than it will ever be exposed to during the lifetime.

Just to demonstrate what we mean by memory and specificity If you inject a mouse with antigen X, after a couple of weeks it will start forming antibodies and then it will break and the the lvl will go down. If after (let's say 7 weeks) injected again with two antigens (one is the same antigen that it had been exposed before and the new antigen) the response to X antigen is typically quicker and more robust and sustained while the response to the new antigen (Y) is the same as the first time response to X antigen. Which means: 1- It remembers the previous exposure. 2-it can differentiate between X and Y (response to Y wasn't affected by response to X).

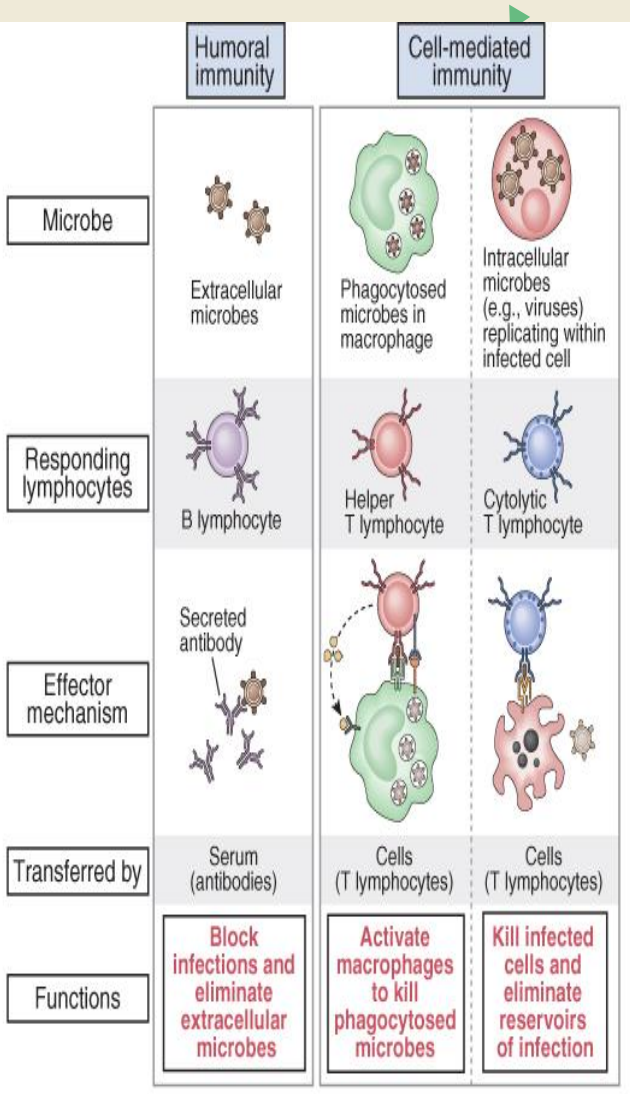


Adaptive system

The **humoral** component of the adaptive immune system is a functional lymphocytes which forms antibodies against extracellular antigens and microbes that can work infections & eliminate extracellular microbes.
The cell-mediated Immunity has two components (and other components):

T-helper lymphocytes: They orchestrate cytokines that are important in the activation or control/ regulation of different responses.
It activates macrophages that already has engulf microbes and stimulates it to kill the intracellular microbes

Cytotoxic (cytolytic):
T lymphocytes: their function is to kill infected cells (mainly viral infected cells) to prevent harm & existence of any reservoirs.



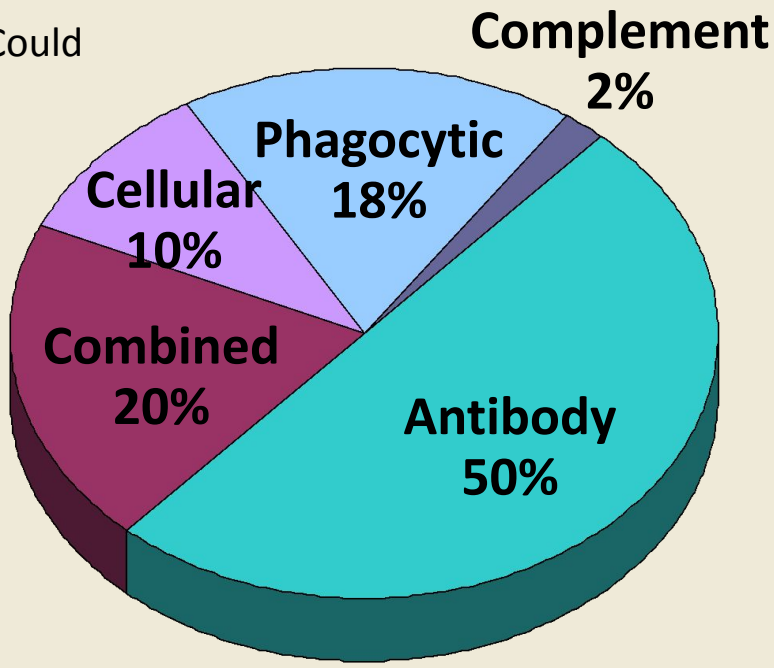
Immunodeficiency

Definition:

- Immunodeficiency represents a diverse group of abnormalities of the immune system resulting primarily in an increased susceptibility to infection (this is the main feature of immunodeficiency diseases).

- Primary Immunodeficiency our subject today: Congenital (inherited). it could affect any part of the immune system
- Secondary Immunodeficiency: Acquired, Could be transient or permanent.

Primary Immunodeficiency: Overall prevalence of clinically significant PID is thought to be about 1 in 2000.
Primary immune deficiency could affect any line of immune system; could affect humoral, cellular (or both) phagocytes, complement or else.
Here in Saudi Arabia the prevalence is significantly higher (maybe 1 in 500).



Primary Immunodeficiency

Introduction:

- Recurrent infections are generally the most common presenting feature of primary immunodeficiency diseases (PIDs). Immunodeficiency should be considered in children who present with **S**evere, **P**rolonged, **U**nusual, or **R**ecurrent infections.

- Certain non-immune related illnesses may present with recurrent infections. For example, CF and ciliary dyskinesia cause recurrent sinopulmonary infections (mucus accumulation or thick mucus that's difficult to get rid of), H-type TE fistula cause recurrent chest infections (aspiration), and VUR cause recurrent UTI. (*) CF/PCD cause recurrent pneumonia.

- Many PIDs are complicated by autoimmunity and malignancy because these things represent dysregulation of immune system pathways.

- Careful history and physical examination can give the most important clues that will direct your investigations and further management.
- Don't forget that not every patient with recurrent infections is having primary immune deficiency (or immune deficiency in general). All of this because of either physiological or anatomical more than of a defect and do not represent immune deficiency just because they have a recurrent infection .

Specific Immune Deficiencies affecting different lines of the immune system:

B-cell immunity deficiencies	Phagocytic defects
Complements deficiencies	T-cell immunity deficiencies

Specific Immune Deficiencies affecting different lines of the immune system

Complement Deficiencies

- Early classical pathway components (C1, C4, C2,C3):
  Defect leads to Pyogenic infections, lupus like illness, vasculitis and glomerulonephritis (autoimmunity).

- Late (Classical) complement components (C5-9):
  Presents with Recurrent or disseminated Nisserial infection especially Neisseria meningitidis. So if a patient has recurred neisseria meningitidis, you need to think of defect in late complement pathway.
- Complements are a cascade of proteins that serves certain functions (lyse bacteria, opsonization which is attracting phagocytic cells to the organisms, augment the humoral immune response). Starts from 1 to 9

Specific Immune Deficiencies affecting different lines of the immune system

Phagocytic defects:

A. Disorders of neutrophil number:

- Cyclic neutropenia and severe congenital neutropenia. It can affect the number (ex. neutropenia by congenital neutropenia) as they are group of defects leading to inability to produce neutrophils. The defects happened in the early development/ stages of neutrophils in bone marrow.

B. Disorders of adhesion:

- Leukocyte adhesion defect[1]. Defects may involve the ability of neutrophils to adhere to endothelium (because when there is inflammation Neutrophil has to go from bloodstream to the tissue (the site of inflammation) it has first to adhere to endothelium, then it goes from blood vessels to the tissue for a process called chemotaxis, then it engulf and kill the bacteria there in that site.

C. Disorders of chemotaxis:

- Chediak-Higashi syndrome. (also have abnormal intracellular killing)

D. Disorders of intracellular killing (ability of neutrophils to kill & ingest bacteria):

- Chronic Granulomatous Disease. Inherited Disorder.

Chronic Granulomatous Disease (it's a classic disease)

- A defect in the enzyme called NADPH oxidase enzyme system leading to failure of production of oxygen radicals and hydrogen peroxide which lead to inability of intracellular killing of mostly catalase + bacteria and fungi.
- Mechanism: it oxidases NADPH & reduced O2 (producing O- aka ROS, which are toxic & are converted to the more toxic H2O2 (hydrogen peroxide) and that can be further converted to HOCl (it is essentially bleach; so toxic to bacteria) NADPH oxidase is composed of different components, a defect in any of them leads to a defect in the enzyme.. one of them is encoded in chromosome X (this disease can therefore be X linked or autosomal recessive)
- Manifest with recurrent pneumonia or deep seated abscesses due to staph aureus (most common), serratia, B. cepacia, aspergillus and other organisms
- Can be inherited as X-linked or autosomal recessive. How? Because it's composed of different components (5 components) one of them is gp91 which is encoded in X-chromosome (defect will be X-linked), the others are in different autosome (defect will be autosomal recessive). So, it depends on where the defect is.
- Diagnosis: NBT or oxidative burst assay[2].
- Management: Patients are put on bactrim and itraconazole prophylaxis.
- BMT (bone marrow transplant) is recommended for some patients. Treatment of choice (Curative)

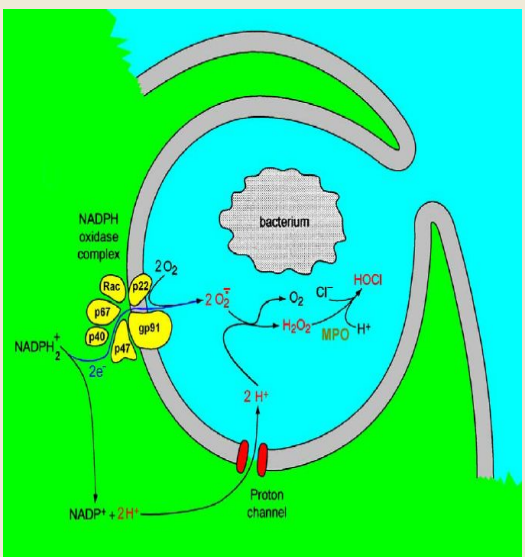
1- They may present with high neutrophils count, inability to form abscesses, delayed separation of the umbilical cord (normally 1-2 weeks; here: could be up to 6 weeks)

2- Demonstrating the inability of the cell to produce reactive oxygen species (ROS) by different tests; NBT is old, flow cytometry is now more used

Chronic Granulomatous Disease Cont,

- When a neutrophil in engulf bacteria inside and I saw the in NADPH oxidase enzyme oxidases NADPH & gives it oxygen so it produces reactive oxygen species (O2-) which are toxic but it could help it killing interested bacteria because it produced inside lysosomes, isolated from other cellular components. But it's further produced into H2O2 (hydrogen peroxide) by another enzyme (superoxide dismutase), hydrogen peroxide is antiseptic it's even more toxic to the bacteria than O2-. H2O2 is converted by another enzyme (Myeloperoxidase) to HOCl (Hypochlorous acid) is bleach basically (like clorox), it's more toxic than H2O2 to the bacteria.
- NADPH oxidase composed of multiple components (multiple proteins chains that together for this enzyme) if there is a defect in one of those components it will lead to the defect in the enzyme therefore inability to produce reactive oxygen species..etc. therefore inability to kill the ingested organisms. Thus, will lead to inability to eliminate the infection bacteria does complicate infection (pneumonia complicated by empyema, bronchiectasis, major abscess formation, sepsis). Those patients at risk of fungal infections (like aspergillus).
- Catalase used to identify the type of certain organisms. The catalase enzyme can catalyzes H2O2 (hydrogen peroxide) And H2O2 can be produced by some bacteria, so those catalase positive organisms can degrade their own H2O2, but catalase negative they can not. So if patient has NADPH oxidase deficiency, the patient can use H2O2 of the bacteria and convert it to HOCl (which is more toxic) and use it to kill bacteria that will give little compensation to the defect. But catalase positive organisms the body can't utilize their H2O2 because they degrade, so those patients mostly affected by catalase positive organisms examples staph. Aureus & Aspergillus.

- How to diagnose chronic granulomatous disease? Simply by demonstrating the inability of neutrophil to produce reactive oxygen species by flow-cytometry (standard method) device that can recognize individual cells labeled by antibodies using different lasers .
- How to manage those patients? -First line management: antibiotics prophylaxis: combination of Bactrim (for different bacteria) itraconazole (for aspergillus) which is the most common cause of death in those patients.Using them will not prevent all infections but it decreases the risk of infection by about 70%.
- Ultimate treatment is bone marrow transplantation (BMT): it -especially in patient with severe disease. will replace patient neutrophils with normal neutrophils from the donor.
- Patient will be able to transmit the disease to the offspring, but will be cured if this procedure succeeded (especially if there is HLA identical match)



Deficiencies in T-cell immunity

The most severe form of immunodeficiency because T cells play a major role in immune defects

❖ Clinical Characteristics:

- Often present early before 5 months of age.
- Usually associated with recurrent infections with fungal, viral, or mycobacterial pathogens bc T cells play a role in defense against all kind of organisms .
- Patients may develop infections with opportunistic organisms. e.g: Pneumocystis jiroveci If you have a patient who has an infection with pneumocystis jiroveci which is an opportunistic organism rarely happens in immunocompetent individuals you have to think of immune deficiency, you can't treat it and let it go (whether it's acquired HIV or inherited) but you have to investigate .
- Severe failure to thrive bc of serious recurrent infections.
- GVHD may develop secondary to blood product transfusion or in-utero from materno-fetal transfusion Graft versus host disease: in graft, immune cells works against the host because the host is immune-compromised. Normally, when immunocompetent takes packed RBCs, the immune system will easily be able to eliminate the few WBCs in the packed RBCs so those few WBCs will not work even if they're considered foreign. But in those patients who are very immune suppressed, they don't have T cells function, so the few white blood cells in the packed RBCs can act against the host leading to GVHD because they're foreign. During pregnancy, some T cells can pass through placenta from the mother to the fetus, and because there is usually HLA mismatch, it will recognize fetus as foreign, therefore it my cause GVHD.
- Often associated with humeral (B-cell) defect because of lack of T-cell help B-cell Function depends partially on normal T-cells function, T-helper cells orchestrate the immune response so they are important to activate B-cells and phagocytic cells.

❖ Causes:

A. Acquired:

a. Severe malnutrition most common cause worldwide.
b. Immunosuppressive drugs like chemotherapy.
c. Radiation (Radiotherapy).
d. Infections: like HIV.

B. Congenital:

Deficiencies in T-cell immunity The most severe form of immunodeficiency

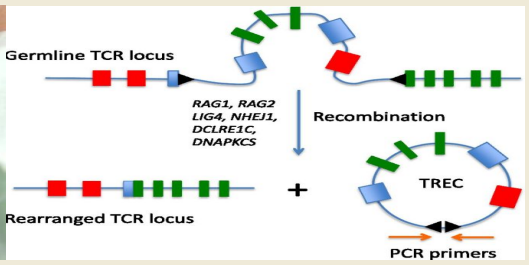
Deficiencies in T-cell immunity

a. Severe Combined Immunodeficiency Syndromes (SCID): Most severe form of immune deficiency (combined because it includes T-cells and B-cells)

- ❖ Usually characterized by marked lymphopenia and very early presentation in the first few months of infancy.
- ❖ Lymphopenia (< 2000/ul) in a healthy neonate is an indication for investigation Even if the patient looks completely healthy, you have to think of SCID because maybe patient haven't got any infection yet.
- ★ Pediatric Emergency Because if you recognize it early you can interfere, you can treat and try to prevent infection. Because those patient will eventually need bone marrow transplantation -as the only curative treatment- and if BMT preformed early before a serious infection developed those patients most of them will be cured. But, if serious infections develop with time the success of BMT will be no significant, it will drop from >95% to <80%.
- ❖ The success rate of stem cell transplantation in the first 3.5 months of life, and before infection develops, is >95% as compared to < 80% if done later with infections.

Newborn Screening for SCID:

- ❖ Since early detection and early BMT for patients with SCID is critical a newborn screening was developed.
- ❖ This screening is based on the detection of T-cell receptor excision circles (TRECs) by PCR, which indicate production of naïve T-cells from the thymus.
- ❖ There is a newborn screening technique, in some places they do it to screen newborns for SCID by using charts (similar methodology of screening in some metabolic diseases, they are slips of papers, they do heel stick of newborn, then they take drops of blood & they send it for screening, & it can screen against multiple metabolic diseases (>17 different inherited metabolic diseases).



b. Combined immune deficiency (CID) Not as severe they don't have severe lymphopenia but they have combined immunodeficiency and clinically they may be as severe as SCID. (The distinction is the number of lymphocyte):

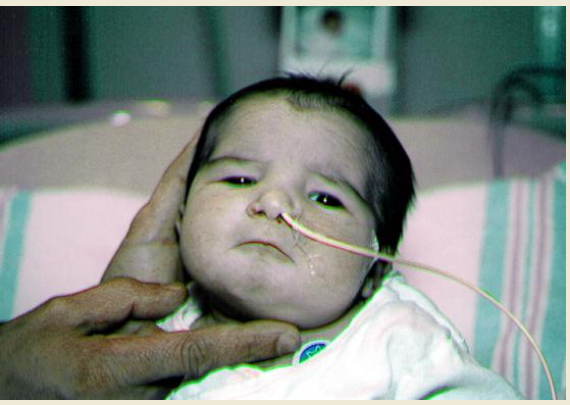
- ❖ T- cells are not severely deficient (> 300/ul) and presentation may be less severe (but not necessarily). Examples:
- ➢ Wiskott-Aldrich Syndrome.
- ➢ Ataxia Telangiectasia.
- ➢ DiGeorge syndrome.

Deficiencies in T-cell immunity

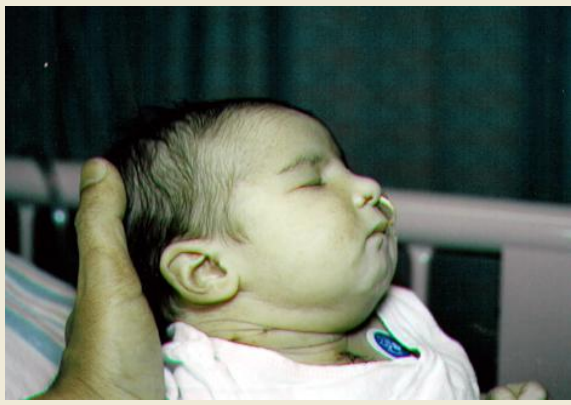
DiGeorge Syndrome:

- Features (mostly in midline) : congenital cardiac malformation involving large vessels, hypoplastic thymus, parathyroid deficiency, velopharyngeal insufficiency, cleft palate, and dysmorphic features.
- Results form a defect in the early embryonic development of the 3rd and 4th pharyngeal arches Because from 3rd and 4th pharyngeal arches comes the heart, thymus, parathyroid and several midline structures .
- The cause is: Most patients have genetically microdeletions affecting 22q11. (q means the long arm)
- Most patients have normal T cell number and functions and most of those with lymphopenia will recover by the end of their 1st year.

- Small fraction of them -especially patients with thymic aplasia- have severe lymphopenia that need thymic transplantation. Most pts will have partial thymic aplasia, temporary low T cell number, but mostly they don't have problems related to immunodeficiency. If they have total thymic aplasia they will have severe T cell problems & will need thymic transplantation

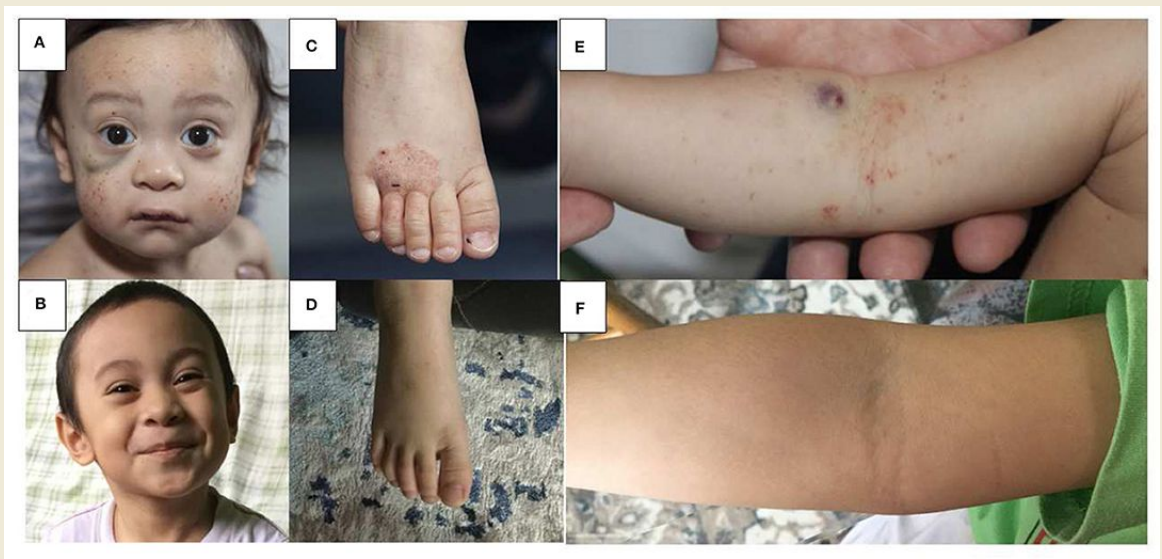


Dysmorphic features: bulbous nose, frontal bossing, sometimes anti-slant of the eye, micrognathia, low set ear, sutures in the chest indicating cardiac surgery. They indicate certain genetic diseases, and needs investigation for genetic defects.



Wiskott-Aldrich Syndrome:

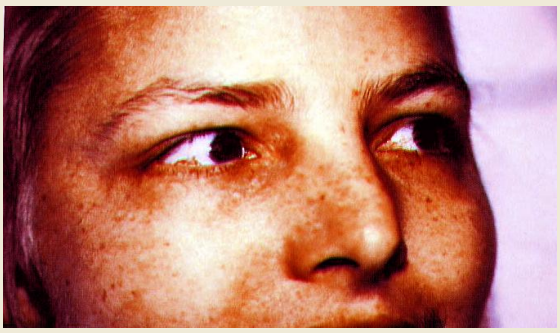
- X-linked disease.
- Characterized by triad of: eczema, thrombocytopenia with small platelets (unlike ITP with large platelets), and combined immunodeficiency.
- Patient's Lymphocyte proliferation is depressed.
- They may have variable antibody abnormalities.
- Treatment is by BMT.



Deficiencies in T-cell immunity

Ataxia-Telangiectasia:

- Autosomal recessive disease.
- Progressive cerebellar degeneration therefore they have ataxia.
- Cutaneous (skin) or ocular (conjunctiva) telangiectasia.
- Immunodeficiency affecting predominantly cellular immunity (T & B cells).
- Sensitivity to ionizing radiation. So we try not to expose them to x-rays unless it's necessary.
- High incidence of malignancies.
- The basic problem is a defect in DNA repair. Because they have a defect called ATM.
- Patients have elevated alpha-fetoprotein as a marker.



Deficiencies of B-cell immunity

Clinical Characteristics: it's milder

- Onset is usually after 7-9 months Later because maternal immunoglobulins gives some protection (mainly IgG) in the 3rd trimester.
- Recurrent infections with encapsulated organisms especially encapsulated bacteria.
- Patients usually develop chronic or recurrent sinusitis, otitis media, pneumonia. They may also develop recurrent sepsis, meningitis, or osteomyelitis.
- Few problems with fungal or viral infections (except enteroviruses and polio)
- Little growth failure.

Major causes:

- X-linked agammaglobulinemia.
- AR agammaglobulinemia.
- Common variable immunodeficiency (CVID): hypogammaglobulinemia with poor antibody responses to antigens.
- Hyper-IgM syndromes: High-normal IgM with low IgG and IgA.
- They have defect in mechanism called class switching. it means naïve B-cell which normally produces IgM as a response to foreign antigens, but after it's exposed to an antigen that activates it, it switches from IgM-producing cell to IgG or IGA producing cell against infectious organisms.
- IgG is more efficient and can control antigen and infections organisms better (especially coordination with other immune cells). So there is a defect in this switch that they can't switch and that's maybe because of defects in several genes leading to this problem.
- Positive IgM = indicates acute infection.
- Positive IgG = indicates chronic or old infection. And that's because of the switch from igM to igG that happens.

Clinical presentation of primary immune deficiency

Defect	Presentation	Examples
T-cell defects	Severe and/or unusual viral and fungal infections and faltering growth in first months of life, e.g. severe bronchiolitis, diarrhoea, oral thrush, *Pneumocystis jirovecii (carinii)* pneumonia (PCP), disseminated or severe cytomegalovirus infection.	**Severe combined immunodeficiency (SCID).** Heterogeneous group of inherited disorders of profoundly defective cellular and humoral immunity. Fatal without treatment. **HIV infection** **Wiskott–Aldrich syndrome** – triad of immunodeficiency, thrombocytopenia, and eczema (X-linked). **DiGeorge syndrome** – with abnormal development of the 5th branchial arch causing cardiac defects, palatal and facial defects, absence of thymus, and hypocalcaemia (deletion of section of chromosome 22; 22q11). **Duncan disease (X-linked lymphoproliferative disease)** – inability to generate a normal response to Epstein–Barr virus; either succumb to the initial infection or develop secondary lymphoma. **Ataxia telangiectasia** – defect in DNA repair, also increased risk of lymphoma. Cerebellar ataxia, developmental delay.
B-cell (antibody) defects	In first 2 years (beyond infancy because of passively acquired maternal antibodies) – severe bacterial infections, especially ear, sinus, pulmonary, and skin infections; recurrent diarrhoea and faltering growth. Recurrent pneumonias can lead to bronchiectasis; recurrent ear infections to impaired hearing.	**X-linked (Bruton) agammaglobulinaemia.** Abnormal tyrosine kinase gene; essential for B-cell maturation. **Common variable immune deficiency (CVID) –** B-cell deficiency. High risk of autoimmune disorders and malignancy. Later onset than Bruton agammaglobulinaemia. **Hyper IgM syndrome** – B-cells produce IgM but are prevented from switching to IgG and IgA. **Selective IgA deficiency** – most common primary immune defect. Usually asymptomatic, or recurrent ear, sinus, and pulmonary infections.
Neutrophil defects	Recurrent bacterial infections – abscesses (skin, lymph nodes, lung, liver, spleen, bone), poor wound healing, perianal disease and periodontal infections; invasive fungal infections, such as aspergillosis. Diarrhoea and faltering growth. Granulomas from chronic inflammation.	**Chronic granulomatous disease** – most are X-linked recessive, some autosomal recessive. Neutrophils fail to produce superoxide after phagocytosis of microorganisms.
Leucocyte function defects	Delayed separation of umbilical cord, delayed wound healing, chronic skin ulcers and deep-seated infections.	**Leucocyte adhesion deficiency (LAD) –** deficiency of neutrophil surface adhesion molecules, CD18, CD11b, leads to inability of neutrophils to migrate to sites of infection/inflammation.
Complement defects	Recurrent bacterial infections. SLE-like illness. Recurrent meningococcal, pneumococcal and Haemophilus influenzae infections – with deficiency of complement components.	**Early complement component deficiency** **Terminal complement component deficiency** **Mannose-binding lectin (MBL) deficiency.**

General Approach to Patients with Suspected Immunodeficiency

❖ Usually, the earlier the onset the more the likelihood of severe immunodeficiency. Age of presentation may give a clue to certain things.

For example:

- SCID (severe combined immunodeficiency) usually presents in the first 4-5 months of life.
- Agammaglobulinemia usually presents at 7-9 months of life.

❖ Family history is extremely important! (Parental consanguinity, family history of immune deficiency, early death in the family).

❖ **Site of infection:**

Involvement of specific sites is likely more common with specific types of immunodeficiency than others. Examples:

➢ Recurrent Gingivitis and skin abscesses => Phagocytic defects.
➢ Recurrent Sinopulmonary infections => B-cell defects.
➢ Recurrent Meningitis => complement defects.
➢ Chronic diarrhea should always raise the possibility of immunodeficiency. The nature of the infecting organism may indicate the type of immune defect. Some patients may present initially only with chronic diarrhea .

❖ **Type of the infecting organism:**

➢ Recurrent viral, fungal, mycobacterial, or opportunistic infections => suggest T-cell defects.
➢ Recurrent infections with invasive encapsulated bacteria (e.g: pneumococcus) => suggest B-cell defects.
➢ Recurrent infections with bacteria of low virulence (e.g: staph) => suggest a neutrophil abnormality.
➢ Recurrent Neisseria infections => suggest terminal complement defect.

❖ **History of Adverse Reactions to Vaccines** **Vaccination history is imp!**

➢ Vaccines can be killed which doesn't cause an infection -because it's killed- it can't in immunocompromised. But, in live-attenuated vaccines in some cases they may lead to an infection with that same organisms of the vaccine in immunocompromised patients. So if a patient comes with infection from live-attenuated vaccine, we have to think of immunodeficiency.

➢ Live attenuated vaccines may cause disease in immunodeficient patients. For example, OPV can cause paralysis in a patient with SCID or hypogammaglobulinemia if he receives the vaccine or exposed to it through vaccinated children who are still shedding the live attenuated virus in their stool. It can affect others (ex. if there is an immunocompromised patient in the house, we shouldn't give the child -who is getting regular vaccine- OPV; because it sheds in the stool therefore it can affect others

General Approach to Patients with Suspected Immunodeficiency

Physical Examination

- Absent tonsils => B cell defect Because tonsils mainly formed of B-cells (there is T-cells but mainly B-cells).
- Absent lymph nodes => T/B cell defect (combined immunodeficiency)
- Lymph Node hyperplasia => CVID, CGD The opposite, also might indicate some immune deficiency disease.
- Absent BCG scar => T cell defect But not necessarily, some normal individuals may not have BCG scar. If the scar is present it does not necessarily mean patient doesn't have T cell deficiency as well.
- Delayed separation of the umbilical cord => Leukocyte adhesion defect (Normally the umbilical cord separates within 1 to 2 weeks, If after 6 weeks it is still there think of Leukocyte adhesion defect).

Some patients develop warning signs, they are not well evidence-based بالخبرة but It helps in identifying children with/may have immune deficiency:
1- 4 or more otitis media (especially in toddlers) within one year.
2-Recurrent infection.
3-Too long use of antibiotics for something that is usually can be treated with short course.
4-Two or more pneumonias within one year.
5-Failure to thrive.
6-Recurrent deep skin or organ abscesses and sepsis.
7-Persistent/recurrent thrush in mouth or fungal infection on skin (by candida) which happens frequently in infants and can be easily treated.
8-Need for intravenous antibiotics for something that usually doesn't need such intervention.
10-Family history.

Assessment of the immune system

There're is general investigations and specific investigations to identify the particular event:

Stage-I — **General non-specific evaluation**

Stage-II — **Evaluation based on the suspected type of immune deficiency**

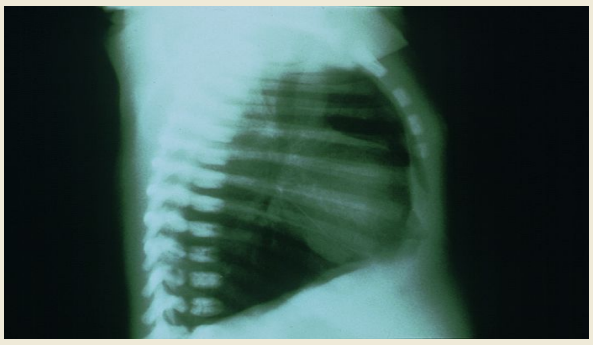
Stage-III — **More detailed investigations**

Assessment of the immune system

STAGE-I: General non-specific evaluation

- CBC, differential (especially WBCs differential, neutrophils, lymphocytes are important. And platelets which are related to some diseases) and blood film.
- Quantitative immunoglobulin levels: Easy test and available everywhere, can give you a quick screen.
- In newborns, CXR for thymic shadow it's not considered diagnostic but if patient has it it raises the suspicion.

Lateral CXR, It normally should have thymic shadow; because thymus in newborns normally is seen (radiopaque), but here there's blackish color.



STAGE-II: Evaluation based on the suspected type of immune deficiency

A. Innate immunity:

- Phagocytic function study (NBT, or oxidative burst).
- CH50 classical pathway, AH50 alternative pathway. Tests to screen the function of complement pathway in general.
- If there is any abnormality they can look at individual component of complement by pathway.
- Flow Cytometry machine for adhesion molecules. It's very helpful in identifying different kind of cells/ markers of cells. Every cell expresses many different molecules on its surface some of the molecules -regardless of their function- can be utilized as markers (they can be 1, 2, 3, combined) to identify the type of this cell; by using antibodies to identify their markers.Those antibodies are labeled by fluorescence, which then detected by a laser in the machine, which gives you the number of cells carrying and not carrying this marker.
- Chemotaxis.

B. Specific immunity:

Humeral component:

- Specific antibodies responses to tetanus, haemophilus influenzae, and pneumococcus.
- If you suspect humoral defect, you can also look for -in addition to immunoglobulin levels in general- specific antibody response to a specific antigen. Example:
  - If someone took vaccine for tetanus, you can check antibodies against tetanus. So, total level of immunoglobulin levels can be normal but specific antibody response can be abnormal. It's qualitative testing (more functional testing).

-Protein antigens and polysaccharide antigens.

- Isohemagglutinins. It's antibodies against AB blood group we used to detect the ability to produce antibodies IgM against polysaccharide antigens
- IgG subclasses. IgG subclasses (1, 2, 3, 4) can also be used in few cases.

Assessment of the immune system

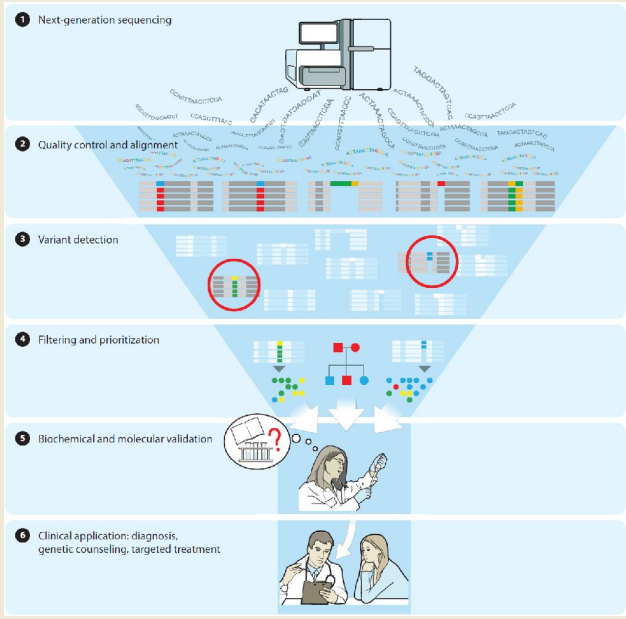
STAGE- III: More detailed investigations

Cellular Component:

- Lymphocyte subsets (quantitative) (CD3,CD4,CD8,CD19,CD16/56)
- CD3 regardless of its function it used as marker for T cells bc it's expressed on T-cells.
- Positive CD3 and CD4 both are marker for T helper cells and used in follow up patients with HIV.
- Positive CD8 and CD3 marker for cytotoxic T cells.
- CD19 and CD20 are markers for B cells
- CD16/56 are marker for natural killer T cells.
- Delayed skin hypersensitivity reaction to intradermal candida or tetanus (not sensitive with limited availability). Rarely used, typical example: PPD test for TB. Induration is T-cell (mainly T-helper) infiltration .
- Lymphocyte proliferation assays in vitro. Standard test, stimulating the cells with certain molecules, then we measure their ability to proliferate.
- HIV testing. Part of differential diagnosis.

Genetic testing is not only in immune deficiency it's in all genetic/ inherited diseases -whatever system involved- by using technology called: Next-generation sequence, which allow us to sequenced the whole genome in 2-3 days and can cost less than $1000. It doesn't give you a diagnosis in 100% of the time, but it became the standard method used in diagnosis.

But more frequently now people are doing whole exome sequencing where they sequence the coding part of the DNA (~2% of it) which is cheaper



From the book

Table 15.3 Investigation to identify primary immunodeficiency – *first line investigations in italics.*

Immune defect	Investigations
Cellular (T cells)	*Full blood count (including lymphocyte count)*
	Lymphocyte subsets (to assess CD3⁺ [total T cell], CD4⁺ [helper T cell], and CD8⁺ [cytotoxic T cell] numbers)
	Ability of T cells to proliferate in response to mitogen
Antibody (humoral; B cells)	*Immunoglobulin levels (IgG, IgM, IgA, and IgE)*
	IgG subclasses (in children >2 years)
	Specific antibody responses (e.g. vaccine-induced antibodies to tetanus and pneumococci)
	Lymphocyte subsets (to assess B-cell numbers)
Combined (B and T cells)	Investigations as above
	Specific genetic/molecular tests for severe combined immunodeficiency
Neutrophils	*Full blood count (to assess neutrophil numbers/neutropenia)*
	Nitroblue tetrazolium test (NBT test) – abnormal in chronic granulomatous disease (most laboratories now use newer assays to determine superoxide production)
	Tests for leucocyte adhesion deficiency – CD11b/CD18 expression
	Tests of chemotaxis (neutrophil mobility)
Complement/mannose-binding lectin	*Tests of classical and alternative complement pathways (CH50, AP50)*
	Assays for individual complement proteins
	Mannose-binding lectin levels
Additional tests	*HIV test*

Management

IVIG in PID

- IVIG (IntraVenous immunoGlobulin) is purified human IgG prepared from pooled plasma of thousands of donors. So it doesn't include all immunoglobulins only the IgG subtypes as it's a normal serum It's extracted from pooled of thousands of donors (at least 1000 donors) therefore it increases diversity, because these different antibodies have different exposures to different antigens.They filtrate it and sterilize it.
- **Mechanism of action:** It is estimated that an IVIG preparation contains ten million antibody specificities. This mechanism leads to:
  - Neutralization of viruses.
  - Opsonization of bacteria.
  - Neutralization of toxins.

- **Indications:**
  - Agammaglobulinemia. As replacement therapy. initially before BMT
  - CVID.
  - CID.
  - we also use it in diseases as an immunomodulator; like in diseases such as Kawasaki, ITP, GBS

- **Dosage:** It is recommended to maintain a trough IgG above lower limit of normal. Infusion is given q 3-4 wks intervals. Why? Because that's the half life of IgG (3 weeks) which is the longest among all other isotypes. There're forms of immunoglobulins IgG that are given subcutaneously every month, every week, or every two weeks.

- Monitoring: IgG trough level q3-6 months.

- Adverse Effects generally considered safe treatment : non-specific generalized reactions are usually reported in 1-10% of patients, mostly mild.

➢ **Mild:** flushing, headache, back pain, chills, myalgia, nausea.
- Intervention: slow infusion and treat symptoms. Like paracetamol and sometimes antihistamines.

➢ **Moderate:** urticaria, bronchospasm, vomiting.
- Intervention: stop infusion and treat symptoms. Then we revise maybe we need to give it more slowly or pre-treat with medication before.

➢ **Severe:** extremely rare: anaphylaxis/anaphylactoid.
- Intervention: stop infusion and resuscitate. Very rare. ? IgG or IgE anti IgA antibodies.

- Organ-Specific and idiosyncratic reactions are rare.
- Risk of disease transmission.

But it has to be a given in a certain way. Start very slow then increase the rate over an hour, until it reaches maintenance those. Then, it should be fixed on that. It takes 3-4 hr.
IVIG is used more as immunomodulator (more than as a replacement) examples:
As the 1st line of Tx in: Kawasaki, GPS, acute ITP,
and in some cases of myasthenia ophthalmoplegia.
As 2nd/ 3rd line of Tx in other autoimmune diseases.

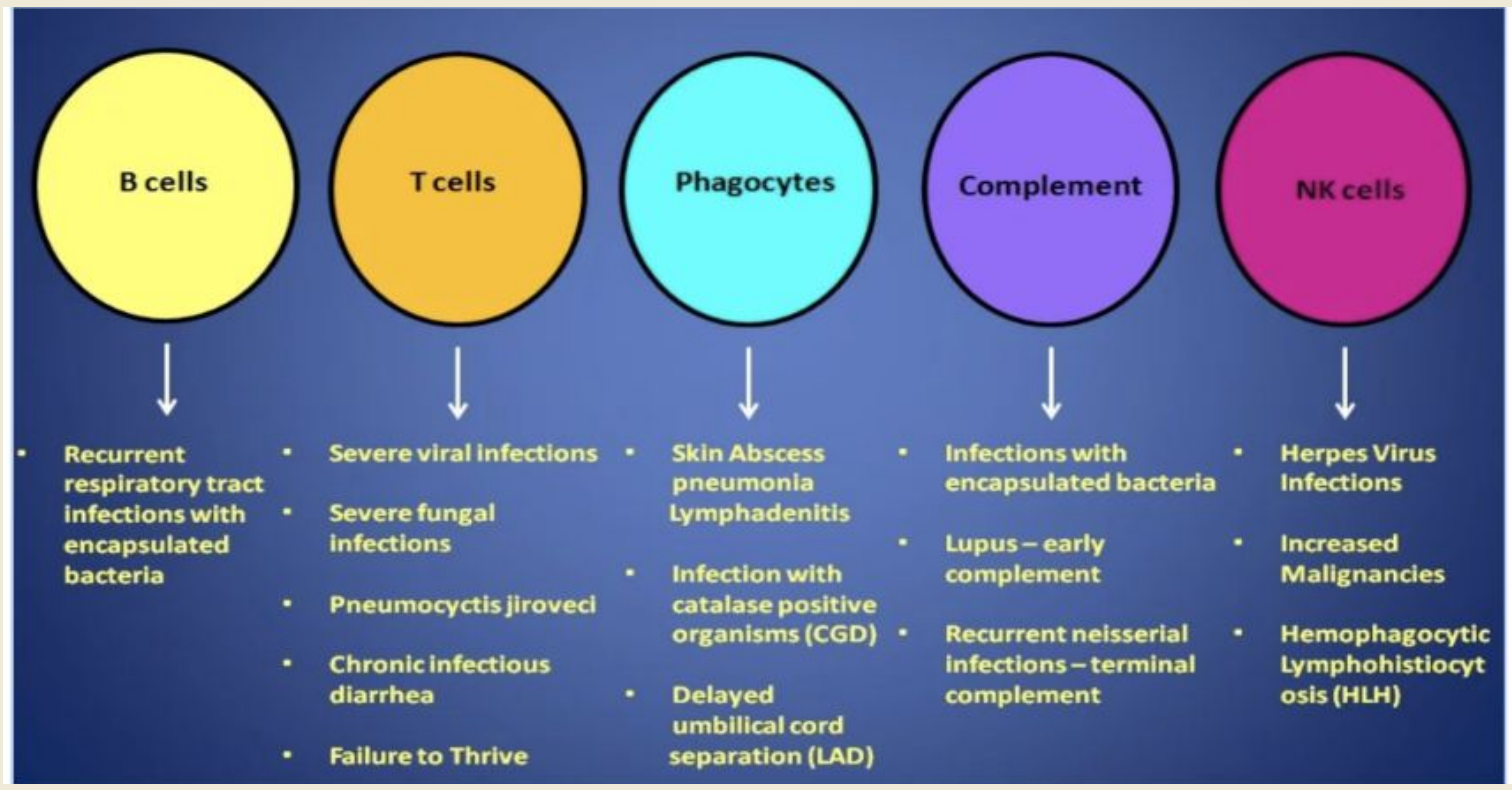
Management

Prophylaxis (book)

- For B-cell defects – antibiotic prophylaxis (e.g. azithromycin) to prevent recurrent bacterial (e.g. chest, ear, sinus) infections
- For T-cell and neutrophil defects – cotrimoxazole to prevent PCP and itraconazole or fluconazole to prevent other fungal infections
- Replacement therapy for hypogammaglobulinemia either for combined or B cell immunodeficiency- initially before BMT
- we also use it in diseases as an immunomodulator; like in diseases such as Kawasaki, ITP, GBS

Management:

- Abx treatment (prompt, appropriate coverage, generally longer courses)
- Screening for end organ disease (ex: bronchiectasis)
- BMT (for SCID, chronic granulomatous disease)



Cases

Case Study-1

An eight-month-old boy was presented to a pediatrician with fever, lethargy, left ocular and facial palsy, and flaccid paralysis of the lower extremities. CSF showed a picture of aseptic meningitis. Two months earlier, the child had received an oral poliovirus immunization.

A presumptive diagnosis of post-infectious polyneuritis was made.

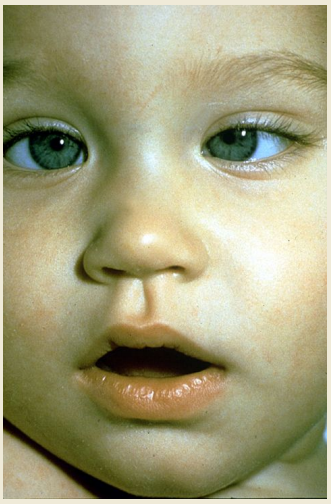
Bulbar palsy: affecting cranial nerves. left ocular facial palsy. flaccid paralysis in peripheral nerves.

What should be done next?

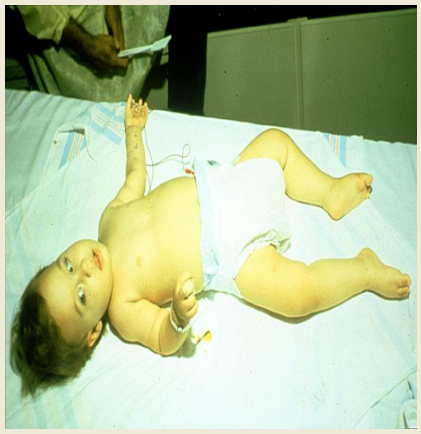
Polio vaccine shouldn't cause polio! So we have to investigate

CBC, Immunoglobulins (same organism OPV cause polio (this make you think that the child may have immunodeficiency like hypogammaglobulinemia or combined immunodeficiency..etc).

Do genetic testing to see what gene is involved



- **Serum IgG concentration was 9 mg/dl (extremely low).**
- **The infant was referred to a pediatric allergist-immunologist.**
- **Mature B- cells were absent from the circulation.**
- **T-cell immunity was normal. The spinal fluid subsequently grew the vaccine strain of poliovirus**. Means what happened was because of the vaccine



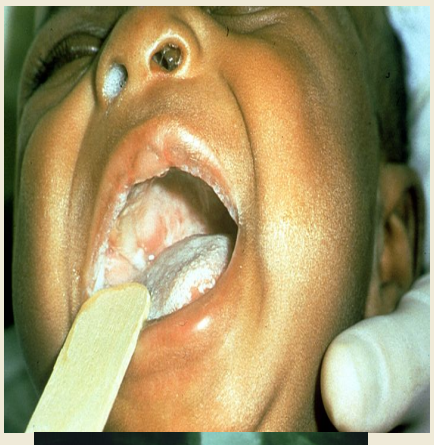
- **Based upon the absence of mature B-cells in the circulation and a state of panhypogammaglobulinemia, a diagnosis of Agammaglobulinemia was made.**

The child has done well on monthly intravenous immunoglobulin replacement therapy, but is hemiplegic

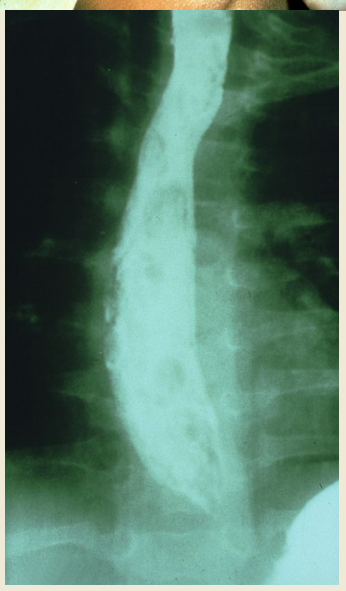
★ **Remember:** Individuals with (or suspected to have) a primary immunodeficiency should NOT be given live vaccines !

Case Study-2

A four-month-old infant was noted to have persistent oral thrush due to Candida albicans. Which doesn't respond to treatment as expected. moreover, the child has extensive mucocutaneous candidiasis involving esophagus.



A consulting immunologist ordered a barium swallow x-ray, and ulcer craters due to this same organism were observed throughout the esophagus . Oral thrush in infants is common but it's not persistent or recurrent in this case, Candida is an opportunistic infection



Where could be the defect?
Combined immunodeficiency

What to do next?
Investigation: Basic CBC, differential and blood film and immunoglobulin levels. Lymphocyte subsets (quantitative) and lymphocytes function (qualitative). Fungal infection —> we suspect the cellular component of the immune system

The child's serum IgG was low (probably maternal), but the IgA and IgM were virtually absent. Few (very low; deficient) mature T-cells could be detected by flow cytometry, and there was no response of peripheral blood lymphocytes to stimulation by mitogens. Means T-cell function also almost absent

A diagnosis of SCID (Severe Combined Immunodeficiency) was made based on the very low T-cell number and their suppressed function.

The child survived with a bone marrow transplantation from his HLA-compatible sister.

Severe defect in the cell number and function and it goes with combined immunodeficiency.