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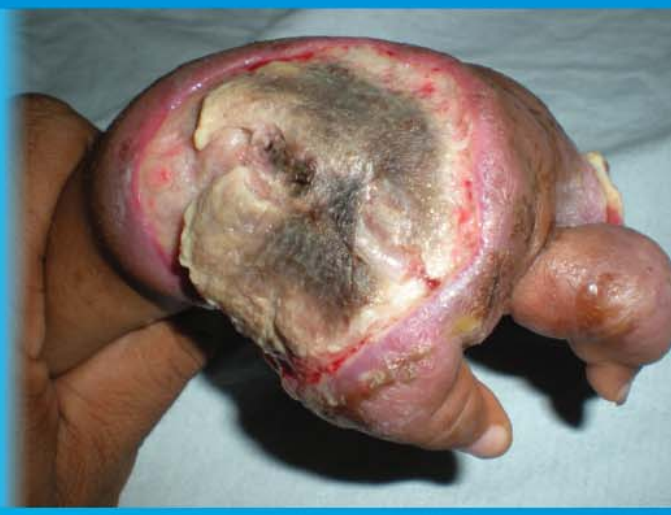
PRIMARY IMMUNO - DEFICIENCY DISEASES (PIDs)



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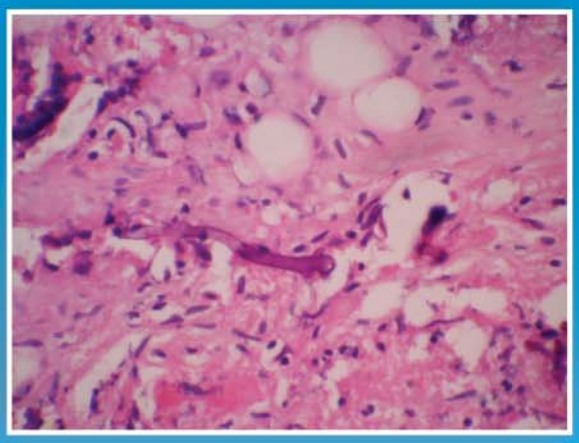
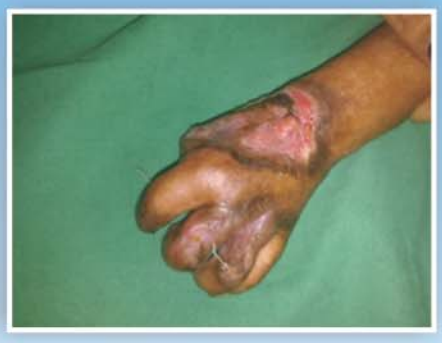


10 month old boy grew Basidiobolus Fungus from an IV site on the dorsum of hand.

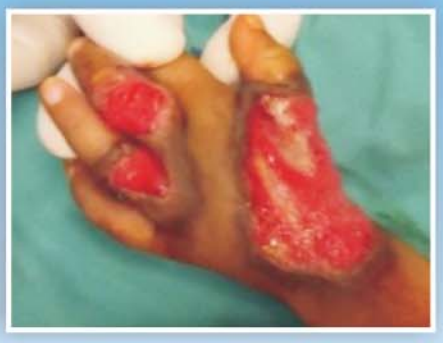
What is the diagnosis? How will you approach this case.

Please e mail to mmdesai007@gmail.com; currimbhoyzinet@gmail.com

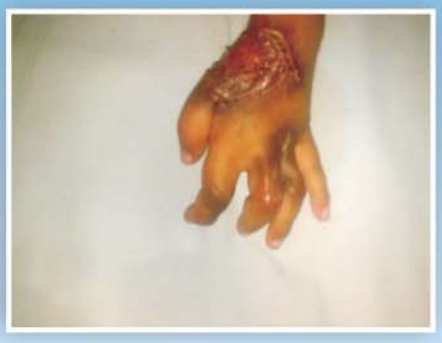
What is the special attraction between a fungus & the exposed parts of the body like skin, respiratory tract & GI tract.?



Pic. 1



Pic. 2



Pic. 3

Sequential pictures of child with IV site infection shown on cover Page

1. After I & D & Amphotericin B treatment.
2. Pre skin grafting; 3. After successful skin grafting.

Primary Immunodeficiency Disorders (PIDs) in Mumbai Infants & Children.

Part I

Aims of this booklet:

Following are the aims of this booklet;

1. Definition of a PID
2. How to suspect a PID: Clinical History & Physical examination will have to be slanted for this purpose.
3. To establish normal laboratory values, in order to evaluate immune-compromised infants and children
4. Analysis of the data obtained clinically, at Bai Jerbai Wadia Hospital for Children (BJWHC) Parel, Laboratory: CBC & Microbiology were done at BJWHC and also at Hinduja Hospital. Immune tests were done at (NIIH) National Institute of Immuno- Hematology, Parel; while tissue histology, immune markers & immunohistochemistry were done at Tata Memorial Hospital, Parel, all very close.

Definition of PID:

Unlike Acquired Immune Deficiency (AID) that is caused by the HIV virus that destroys the CD4 immune cell, PIDs are inheritable genetic disorders that disrupt immune cells either quantitatively or qualitatively in the performance of their functions.

Consequences of PID:

1. Clinical: repeated, persistent or unusual infections and at unexpected ages.
2. Autoimmune phenomena e.g. diabetes in infants, or neutropenia
3. Cancer: dysregulated immune cells are unable to check excessive growth of susceptible cells, or other cells, susceptible to transformation. In 50% of PID children the cancer is lymphoma

Clinical:

It is believed that in third world countries, where adverse environmental conditions exist viz. overcrowding and poor sanitation, it encourages constant exposure to pathogenic microbes early in life, & results in multiple infections. But, it is also believed that that equips us to combat infections later. These concepts need a second look, for several reasons:

- The environment of an infant < 1 year of age is mainly confined to the mother. She holds the baby, cuddles him and breast feeds him & occasionally goes out of her house with her baby. Yet we encountered at BJWHC fever and lumps that frequently start within a few days of life e.g. superficial subcutaneous abscesses in infants 0-2 mths of age due to Staphylococcus aureus, both MSSA (Methicillin Sensitive) and MRSA (Methicillin resistant). MRSA & MSSA can occur at the same time in the same baby in 2 different abscess sites i.e. it is a unique environmental strain

of S. aureus. Initially, we performed NBT, IgE and CH50 tests and when we gathered enough cases and analysed them we realized that most of the babies did not have a PID. We stopped doing these tests unless more signs & symptoms suggestive of a PID were present.

- Host nutritional status is important; fault may not lie only in the environment but in the host as malnutrition may itself make a child susceptible to infections.

Classification of the Immune System:

- Immunity is divided in to Innate & Adaptive immune system.
- **Innate Immunity:** Innate response to an infection is immediate and nonspecific. It is mediated by Neutrophils, Monocytes- Macrophages (Mϕ), NK cells and complement proteins.
- **Adaptive Immunity:** The adaptive response to infection takes time but is specific for that particular infection. It is mediated by B cells, T cells or combined B & T cells (lymphocytes) and they also generate immunologic memory (Fig. 1). A memory response to the same organism is quicker. T or B cell response by T-cell receptor (TCR) and B-cell receptor (BCR) recognizes different epitopes (Antigen / Ag) on the organism. “Immunologic memory” permits the response to be quicker & better because that organism has previously been encountered. The adaptive system can be Humoral (B-cells), or Cellular (T-cells) or both. In reality these T & B response are functionally interdependent, and B cell response may be TD (T cell dependent) or TI (T cell Independent). T-cells respond to viral infections, intracellular organisms, opportunistic organisms & tumors; B-cells do so to Staph aureus, Pneumococcus, Haemophilus. Complement helps B cells & its lack leads to Neisseria meningococcus infection. Therefore all systems co-ordinate to give the best effective response e.g. Macrophages (Mϕs) ingest mycobacteria and secrete cytokine IL12/23 which goes to the T & NK cells and makes T-cells (Th 1 cells) secrete their product interferon gamma (IFNγ), a cytokine, to activate the Mϕ / Mononuclear cells to destroy the mycobacteria.

  DCs (Dendritic cells) and Mϕ present Ags (bacteria, viruses, fungi & parasites) to T/B cells, and are included in adaptive immunity.

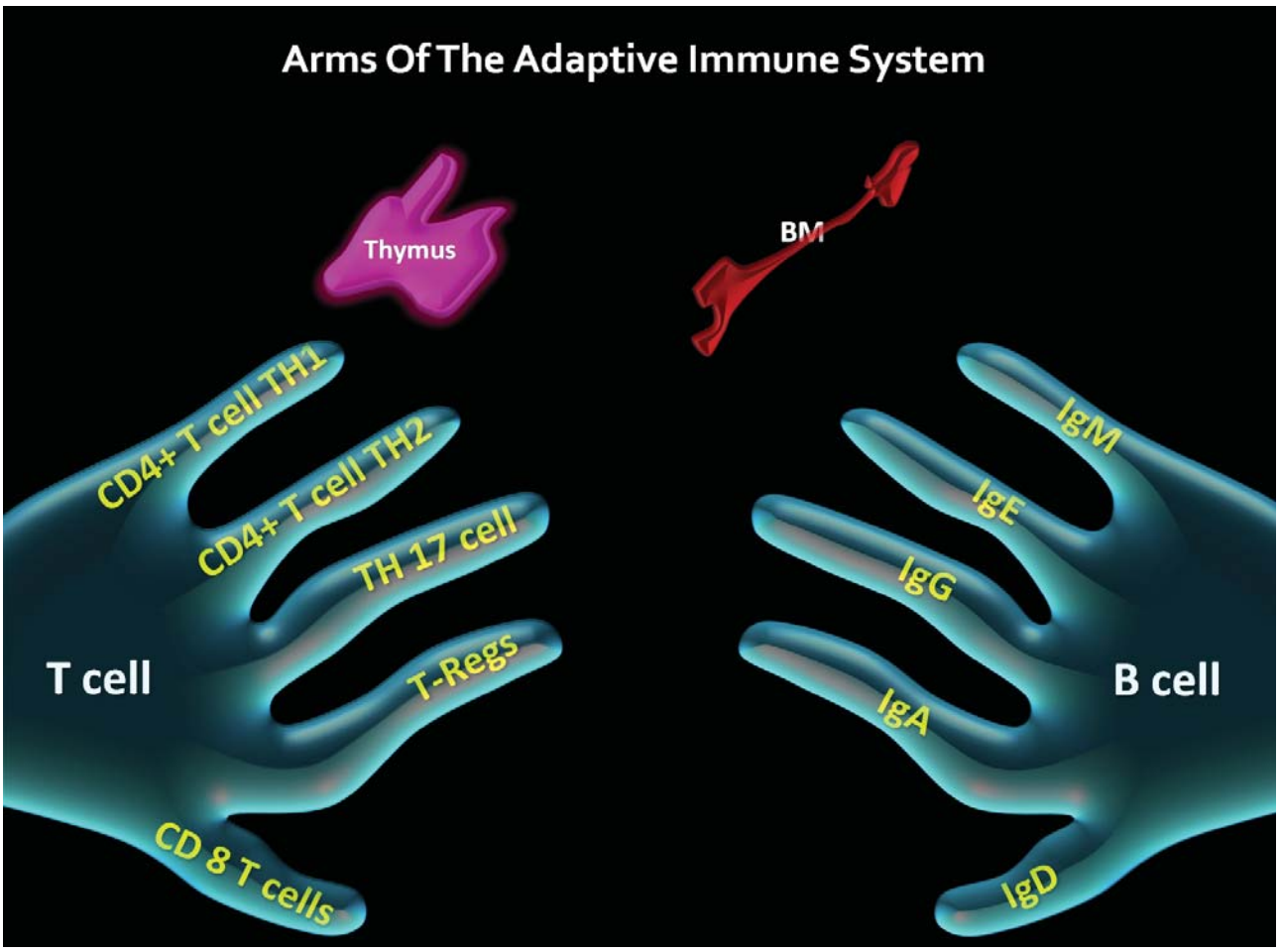


Fig. 1: The arms of adaptive immune system are mainly T cells & B cells. In most situations these arms of the immune system respond in an integrated manner to meet any infectious challenge.

Incidence & Classification of PIDs.

Type of immune defects & their frequency in Europe & BJWHC are shown in fig. 2 & 3 respectively

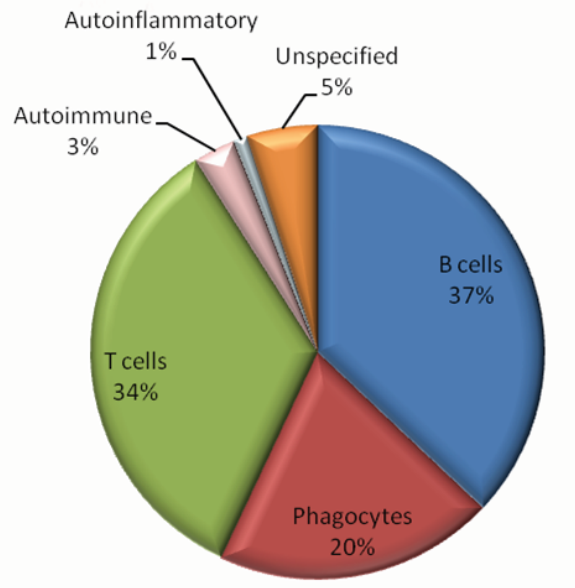


Fig. 2: Type of Defect & Frequency of PID in EUROPE

Prevalence of PIDs French national registry for PID: ESID 11/2008, page 202, Abstract 13; & pages 133 & 134.

In 4/2008- 3/2009 (1 year), we studied 226 suspected cases of PID: 118 (52% had no immune defect) while 109 (48%) had and the break up is as shown in Table (1) & Fig. 3

Table 1: Breakup of type of immune deficiency at BJWHC

Poly	NK	B cell	T cell	Complement	HIES
79	15	5	8	1	1

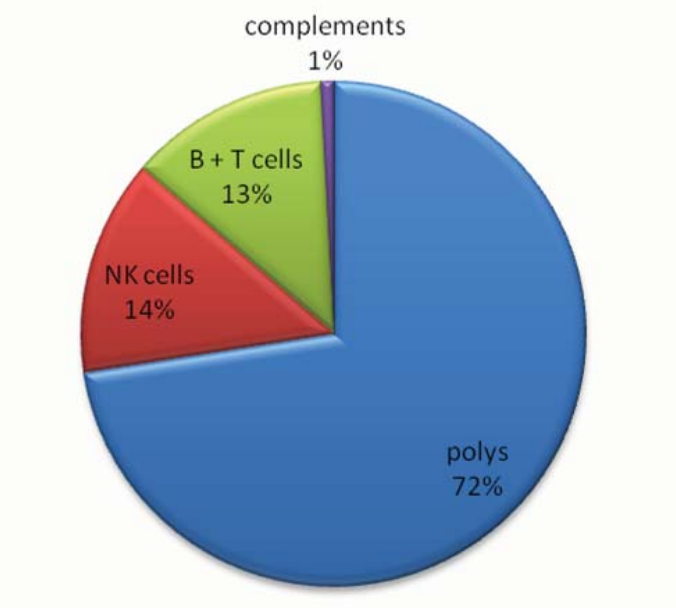


Fig. 3: Type & Frequency of PID at BJWHC

At BJWHC we overwhelmingly have more of innate (Neutrophils) defects followed by NK cells probably because neutrophils respond very quickly to any infection and especially to bacterial infection e.g. Staph which is the commonest pathogen in our lab at BJWHC both in non PIDs and PIDs in the early weeks of life.

PIDs are divided in to 4 compartments based mainly on which immune cell is involved: See Fig. 2 & 3

(a) Phagocyte:

- **Neutrophils:**
  - i. Increased numbers: LAD (Leucocyte Adhesion Deficiency)
  - ii. Decreased numbers: SCN (Severe Congenital Neutropenia)
  - iii. Functional defect: CGD (Chronic Granulomatous Disease)
  - iv. Type of organism: Abscess forming pathogens (Staph. aureus)
- **Monocyte / Macrophage (Mϕ):** Resistance to killing of Mycobacteria by Macrophage is due to defect in IL12/23 - Interferon γ axis.
- **Natural killer (NK cells):** NK & CD 8^+ cytotoxic T-lymphocytes (CTLs) kill viruses, and tumors. Defects in their function result in HLH. (discussed later)

(b) Serum complement: Classical, Lectin, and Alternative pathways are rare causes of PIDs.

(c) B-cell defects: B-cells mature into plasma cells which produce immunoglobulins (Antibodies) to contain an infection or prevent it. XLA (X linked agammaglobulinemia), & CVID (Common Variable Immune deficiency) are examples of B cell defects.

(d) T-cell defects: T-cells deal with viruses, tumors, intracellular pathogens, and opportunistic organisms. e.g. SCID (Severe Combined Immune deficiency)

Knowing the organism often provides important clues to the type of Immune deficiency in a patient Fig. 4 lists the microbial pattern in various types of defect.

With the tremendous help of P D Hinduja Hospital and our lab. at BJWHC, we can isolate most of the pathogens listed in Fig. 4. We are getting vigorous about obtaining cultures: BAL, Biopsy of tissues etc

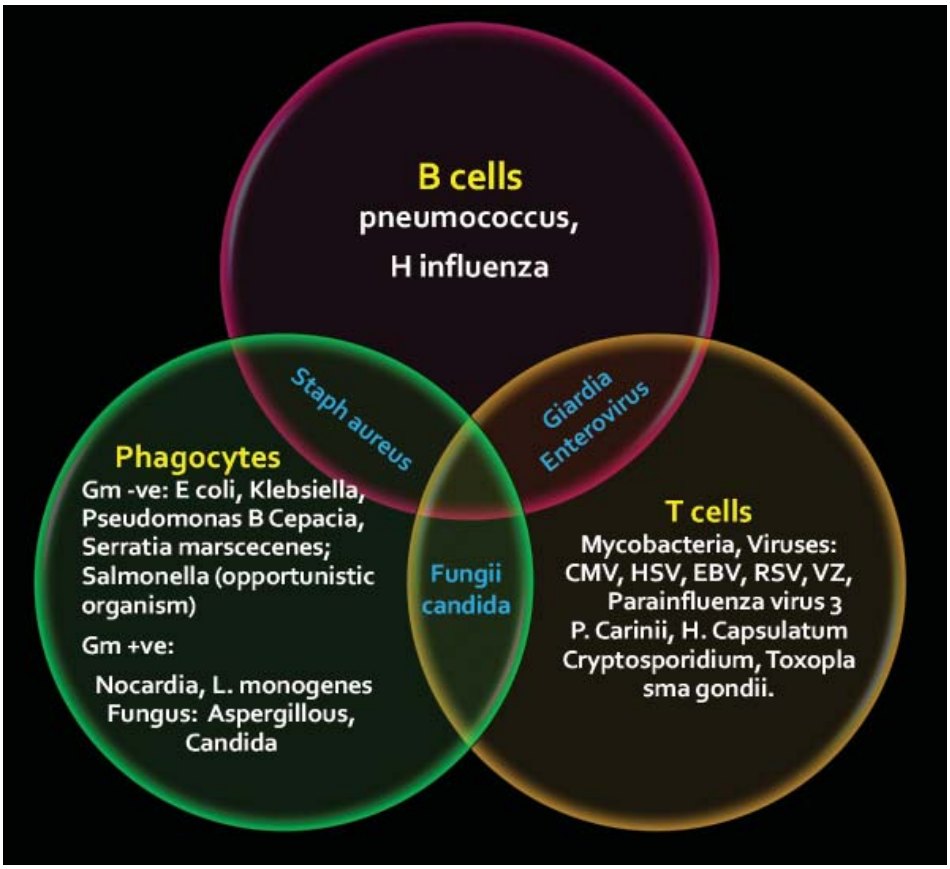


Fig. 4: Microbial pattern in different type of Immune deficiency. Knowing the organism can thus provide important clue to the type of underlying immune deficiency in a patient.

Physical Examination to raise suspicion of PID:

We have 3 exposed mucocutaneous areas.

1. Skin,
2. Sino - Pulmonary system (Respiratory system),
3. Gastrointestinal system (GI tract)

Presenting signs & symptoms

Signs & symptoms which alert one to the possibility of PID are

Skin:

Severe eczema, erythroderma, alopecia, silvery hair, albinism, mouth & anus mucocutaneous candida, dystrophic nails, and pustules.

Sinopulmonary:

Otitis media, sinusitis upper & lower respiratory tract infections (pneumonia) after the age of 6 mths in B cell defects.

GI tract:

Persistent diarrhea due to opportunistic organisms e.g. Giardia, Cryptococcus, Cryptosporidium, Clostridium difficile. How prevalent are they in the general population and also in which areas needs to be worked out. At BJWHC we encounter cryptosporidium more frequently than the others.

In addition to routine physical examination, look specifically for vital signs, age and sex, recurrent oral ulcers, BCG scars, hepatosplenomegaly, enlarged lymph nodes, absence of tonsils, adenoids and spleen as well as dystrophic facial features, Serial growth charts, not only, to assess failure to thrive (FTT), but no gain in weight, especially loss of weight, must be kept.

Laboratory evaluation in suspected PIDs:

Laboratory:

- "CBC", calculate
  - Absolute neutrophil count (ANC) for neutropenia
  - Absolute lymphocyte count (ALC) at birth and later
    - (we are in the process of doing this at different ages and will publish it in the next few months)
  - Platelet count including mean platelet volume (MPV)
    - Needed for – Wiskott Aldrich Syndrome (WAS).
  - Peripheral blood smears for granules (CHS – Chediak Higashi Syndrome)
- Skin test: Mantoux test (MT) to detect a mycobacterial infection whether it be a non TB or Tuberculous mycobacterium.
- CXR: To look for a thymus and lung infiltrates;
- Further tests as indicated.
- Immune tests are done at NIIH (KEM), TMC (Tata Memorial Centre) & few at BJWHC, all located within a few feet of each other. See Fig. 5 for a structure of PID care evolved at BJWHC

NIIH: e.g. Lymphocyte Subset Analysis (LSSA) by flow cytometry, CD18, perforin, DNT (Double Negative T) cells, BTK (Bruton's Tyrosine Kinase), SAP, NBT (Nitro BlueTetrazolium) + DHR (Dihydro Rhodamine), others.

TMH: e.g. Biopsies for malignancies, lymphocyte subsets & immunohistochemistry.

BJWHC: e.g. BM aspirates for hemophagocytes, PB for granules & Asplenia. CH50 (ELISA) for complement and EBV PCR.

One important clue to an ID (Immunodeficiency) is ALC (obtained from, all present-day CBC machines): an ALC of <2000/mm^3 in cord blood or in an infant an ALC of < 1500/mm^3, <20% CD 3, & severe hypogammalobulinemia (IgG <150mg/dL) implies SCID (Severe Combined Immune Deficiency). SCID is a pediatric emergency, resulting in death unless transplanted with stem cells or Bone Marrow (BM).

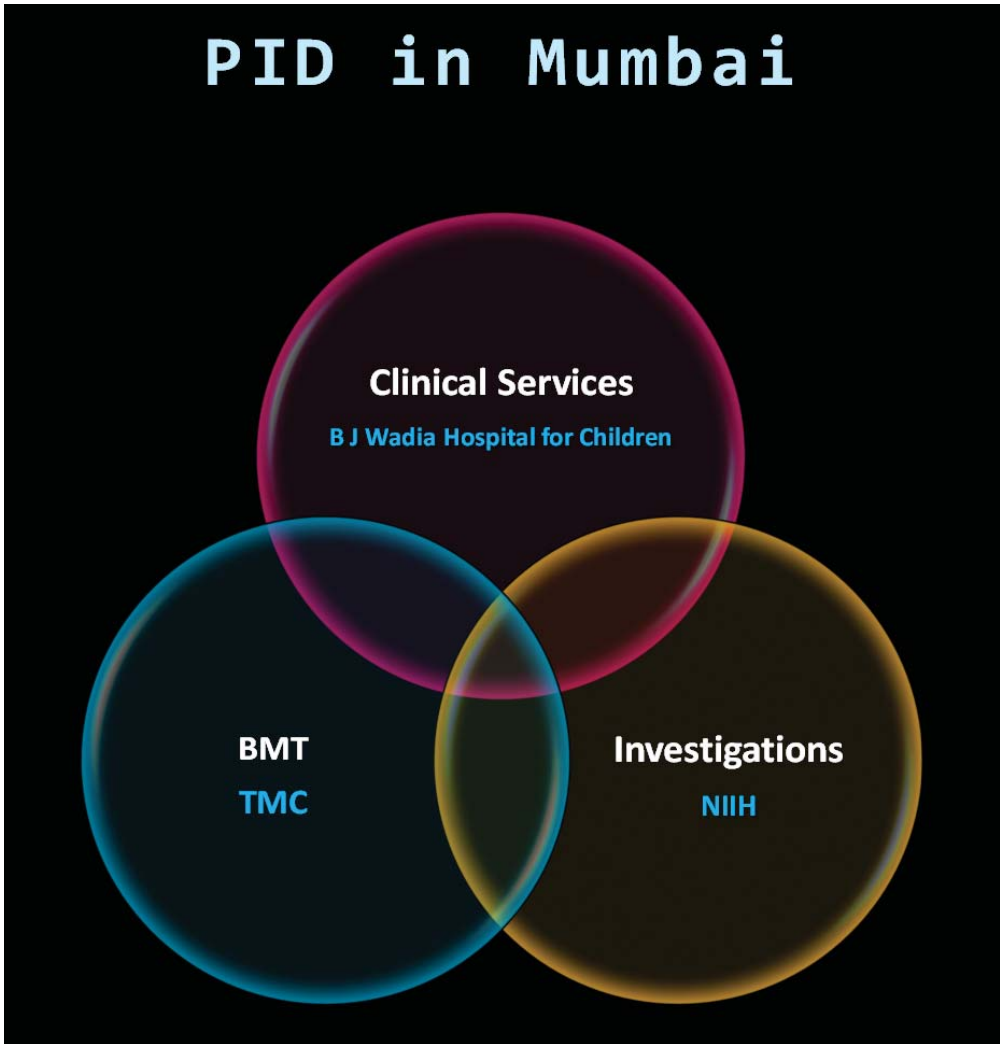


Fig. 5: Structure of PID care in Mumbai. We have evolved the following collaborative structure for Immunology at BJWHC. We at BJWHC provide for the clinical care of PID. At NIIH Investigations are done, with TMC for doing BMT.

In the past 1 year, 280 immune tests were done on suspected PIDs:

Immunoglobulins 130, LSSA (Lymphocyte Subset Analysis) 51, NBT 83 & Perforin 14, and a few more and the diagnosis was verified in 24 (a yield of 11.7%) as follows: Our break-up of current PID problems in 37 babies are

HLH (Hematophagic Lymphohistiocytosis) =15 (HPs on BM), CGD (Chronic Granulomatous Disease) = 9 (NBT, DHR), SCID = 5 (LSSA), CHS (Chediak Higashi Syndrome) = 3 (PB smears), XLA (X linked Agammaglobulinemia). = 4 (CD 19 & CD 20 absent) & ALPS (Autoimmune Lymphoproliferative Syndrome) 1 (DN-ve CD3+, CD4-, CD8- TCR$\alpha\beta$ + cells).

In this booklet, we will not discuss cytogenetic advice needed by parents regarding birth of their next child, prognosis, therapy (both prophylactic to prevent infection and therapy of current infection, genetic therapy & stem cell transplantation).

Few points are very important while treating cases of PIDs:

- Pre transfusion, we need to irradiate blood & blood products when given to a suspected or proven T-cell defect and preferably to all infants below the age of 1 year.
- Do not immunize with live virus vaccines MMR, Polio Vaccine (OPV), Varicella, MR, Measles & Rota virus.
- An ID patient can be given killed vaccines like HAV & conjugate vaccines (Hib, Typhoid, HBV & Pneumococcal) which are T cell dependent. Purified unconjugated Pneumovax is T cell independent.

Part II:

PIDs encountered and their characteristics in BJWHC infants and Children:

We will start with the 4 most frequent problems we saw in the past year: HLH (Hemophagocytic Lymphohistiocytosis), CGD (Chronic Granulomatous Disease), Staph. aureus abscesses; and BCGosis complications with BCG vaccination.

HLH (Hemophagocytic Lymphohistiocytosis):

Case 1:

SG, a 6 month female born of a third degree consanguineous marriage was admitted for fever for 6 days, respiratory distress and an enlarged abdomen noticed for the past 2 days. On physical examination, her weight was 5.25 kg, Ht 59 cms. She was febrile, liver 4 cms and spleen 2 cms were palpable.

CBC: Hb: 5.2 g/dl, WBC 1, 700/mm^3, Absolute lymphocyte count (ALC) of 1632/mm^3, Absolute neutrophil count (ANC) 35/mm^3 & platelets were decreased.

As there was severe pancytopenia, a BM was done which showed hemophagocytes. (Pic. 1)

In HLH, we look out for manifestations of Lymphohistiocytic infiltration in the CNS, Liver and the Bone Marrow.

In SG serum bilirubin was 3.3 mg/dl (direct 1.7 mg/dl), SGPT 78 U/L. Her clotting parameters were deranged with PT 38.2" / 12" control and PTT > 2 mins / 30" control, D-Dimer was 4.42 suggesting disseminated intravascular clotting (DIC).

Dengue fever was also suspected as the USG abdomen showed moderate ascites, minimal pericardial effusion & Rt pleural effusion. She was started on HLH 2004 protocol; however she had a rapid downhill course and succumbed to her illness.

Her perforin levels were done and it was zero; she was a case of Familial Hemophagocytic Syndrome (FHL) with inherited deficiency of Perforin.

We have, over the past one year, picked up 5 other cases of HLH with zero perforin levels.

- Diagnosis of Familial HLH is easier if there is a positive family history. We saw a 12 day old baby born of a third degree consanguinous marriage whose previous 3 siblings had died in infancy. Her perforin was 0.5%. She was diagnosed when she was asymptomatic and we were planning to do BMT (Bone Marrow Transplantation) as that is the only curative treatment. Unfortunately, she developed a mild running nose, severe pancytopenia, hepatic dysfunction, coagulopathy, organomegaly, respiratory distress and altered sensorium. She had all the clinical & laboratory hallmarks of HLH & succumbed to her illness with BM failure.

HLH Case 2:

R., a 5 years old boy, was admitted for fever for 7 days & vomiting with diarrhea for 3 days

He was diagnosed as JRA (Juvenile Rheumatoid Arthritis) 6 months ago, and received weekly injections of methotrexate for 2 months after which it was discontinued by the parents.

He was febrile, BP 90/50 mm and he had swollen knee and elbow joints. Liver 4 cms & spleen 5 cms were palpable.

Laboratory work up was as follows: CBC: Hb 4.2 gm/dl, WBC 20,000 / mm^3; Poly 89%, Lymphocyte 11%, Plts 8000/mm^3.

LFT was abnormal with SGOT 735 IU/L, SGPT 134 IU/L. His LDH was markedly increased at 15,600 IU/L and there was renal involvement with serum Cr. 3.2 mg/dl, an unusual complication.

B.M Aspirate was cellular with many Hemophagocytes.

Diagnosis was HLH, Macrophage Activation Syndrome (MAS) in a case of systemic onset JRA

Furthur specific work up for HLH was done & serum ferritin was a whopping 100,000 ng/ml (values should be at least over 500 ng/ml), Triglycrides (TG) 338 mg/dl (at least > 265 mg/dl), Fibrinogen: 147 mg/dl (Normal > 150 mg/dl), D-D (D-dimer) was elevated & CSF was normal.

In HLH; 5 criteria are important for diagnosis: fever, cytopenia (2 or 3 blood cell lineages); decreased fibrinogen, increased ferritin and Hemophagocytes in BM or Spleen or LN (Lymph Nodes).

HLH can be either

(1) **Inherited FHL:** Familial HLH (types I, II, III, IV); Albinos (Griscelli Syndrome, Chediak Higashi Syndrome) (Pic 3), and X linked Lymphoproliferative Syndrome (XLPS) Fig. 6.

(2) **Acquired HLH:**

a) IAHS (Infection Associated Hemophagocytic Syndrome) especially viral, Leishmaniasis (Pic 4) or after Measles vaccination,

b) MAS (occurs in 17% of JRA)

c) Malignancies (we saw a 2 day old baby with congenital leukemia & peripheral blood hemophagocytosis) (Pic. 2)

Our commonest HLH is acquired HLH due to infections, particularly due to viruses (VAHS). However, in all the above conditions, the trigger for HLH often is an infection (viral). In JRA, the triggers are virus, MTX, gold and NSAIDs.

CD8+veT-lymphocytes and NK cells are recognized morphologically by presence of granules. These granules contain perforin & Granzyme B which are important for inducing death (apoptosis) in targets like virally infected cells. When they recognize the virus or tumor cells they produce cytokines (IFNγ), adhere to their target (the infected cell) and form an immunologic synapse. Perforin pierces a hole in the target cell and Granzyme B granules initiates death by activating the Caspases (enzymes) that cause death. See legend accompanying Fig. 6.

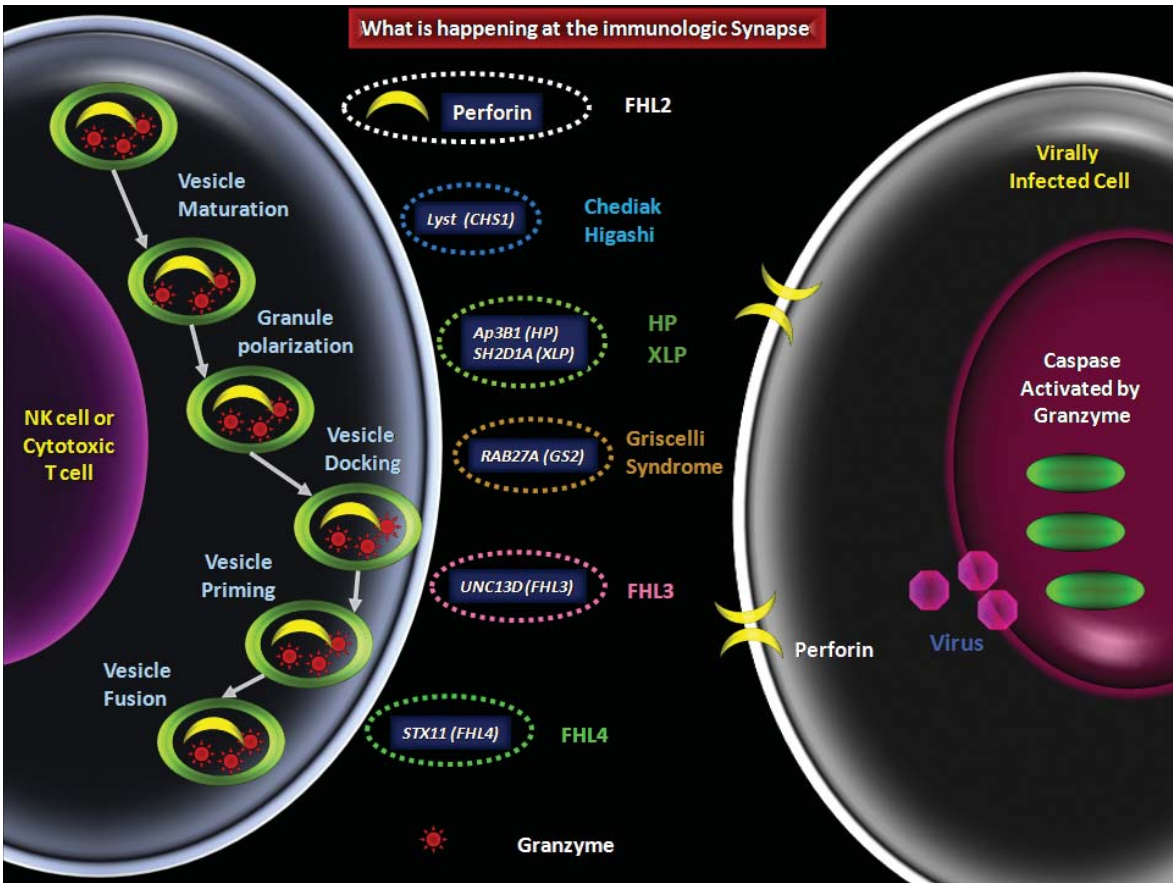


Fig. 6: Depicts the events occurring at the immunologic synapse when a cytotoxic T Cell (CTL) or NK cell identify a virally infected cell for kill.

Initially, an immunologic synapse is formed between the CTL / NK cell and the target cell followed by process of granule exocytosis with release of Perforin & Granzyme. Perforin is a protein with a structural similarity to terminal component of complement hence it perforates the target membrane and forms a channel allowing granzyme to enter and initiate apoptosis of the target cell.

Steps of granule exocytosis along with proteins required at each step with their defects are given below:

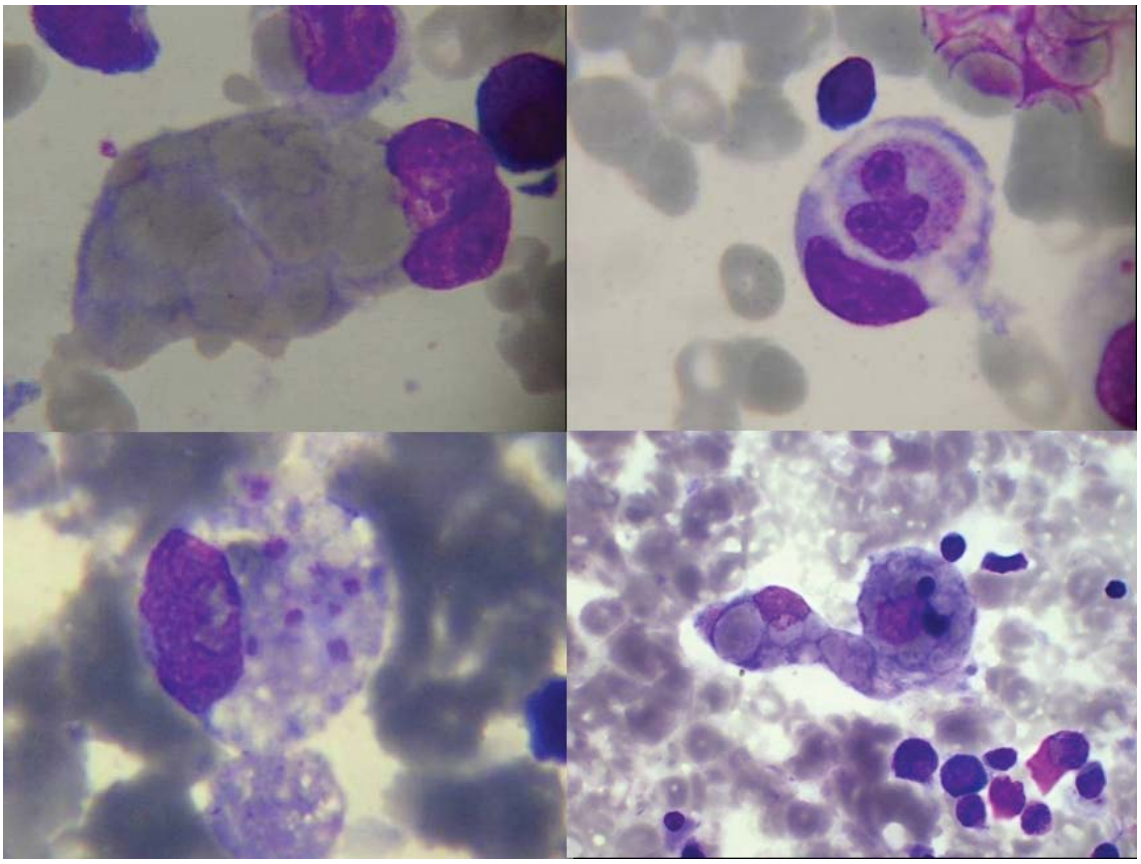
1. Maturation of cytotoxic granule (LYST protein - Disease: Chediak Higashi Syndrome)
2. Granule polarization (Ap3B1 – Disease: Hermansky Pudlak); (SH2D1A; SAP – Disease: X linked Lymphoproliferative Syndrome)
3. Vesicle docking (RAB27α - Griscelli Syndrome)
4. Vesicle priming (UNC13D - FHL3) (Familial HLH)
5. Vesicle Fusion (STX11 - FHL4).

Defect in Perforin results in FHL2

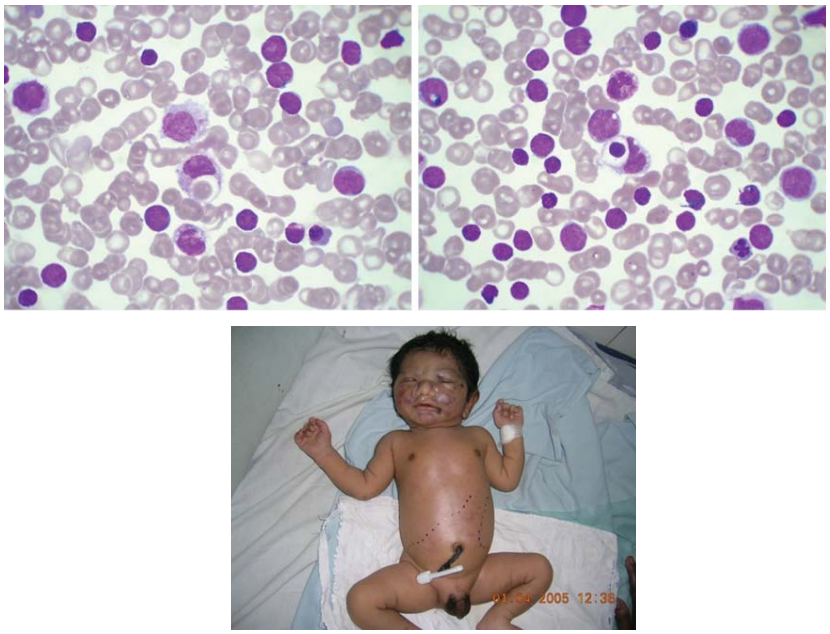
The secretion of the granules is impaired in HLH. Excessive IFNγ activates Mϕs, as is evidenced by hemophagocytosis, & IFNγ is responsible for the clinical symptoms associated with HLH. When NK cells and CTLs cannot kill all the target cells, proliferation and accumulation of these cells result in lymphohistiocytic infiltrates found in the organ see Fig. 7. (Pic 28) (on page 54) show photomicrographs of BM in a case of Chediak Higashi with hemophagocytosis. In Chediak Higashi syndrome the accelerated phase is complicated by Hemophagocytosis. (Pic 3) & the granules are not functional.

In inherited HLH (familial HLH), the age of onset is < 1year in 70-80% and in 75% of these babies, neurological signs and symptoms occur. Hence, we now treat all patients with or without signs of CNS infiltration on admission to prevent it and to prevent relapse.

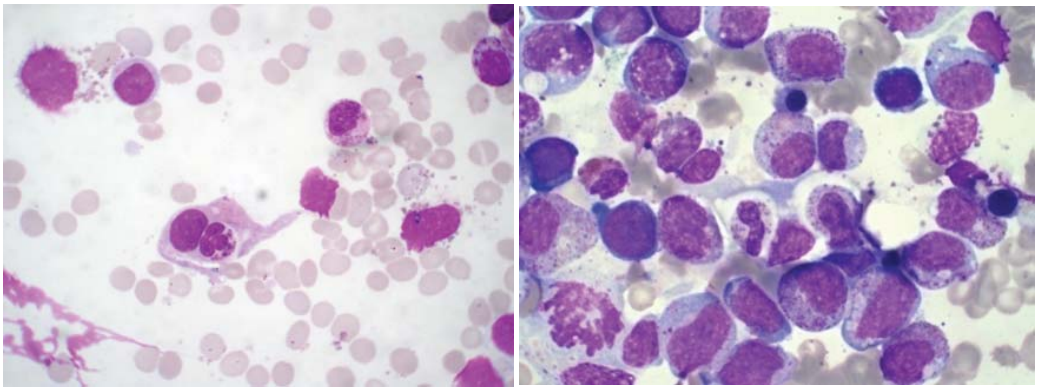
Conclusion: 5 different Inherited syndromes can give the same final result; HLH.



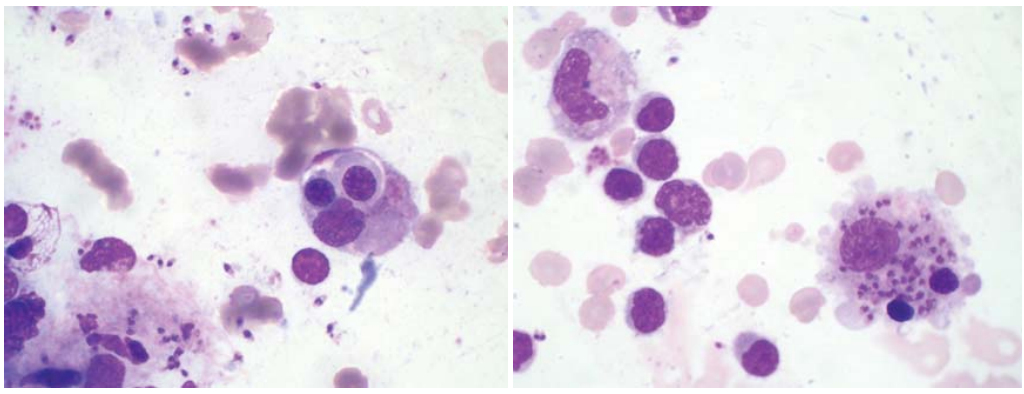
Pic.1: Show BM with erythrophagocytosis, Neutrophil phagocytosis, Platelet phagocytosis & erythrolymphophagocytosis



Pic. 2: A case of congenital leukaemia with peripheral blood HLH



Pic. 3: Photomicrograph of a case of CHS (Chediak Higashi Syndrome) with HLH of Pic 28 (pg. 54)



Pic. 4: Photomicrograph of a case of Kalaazar with HLH an example of IAHS (Infection Associated HLH).

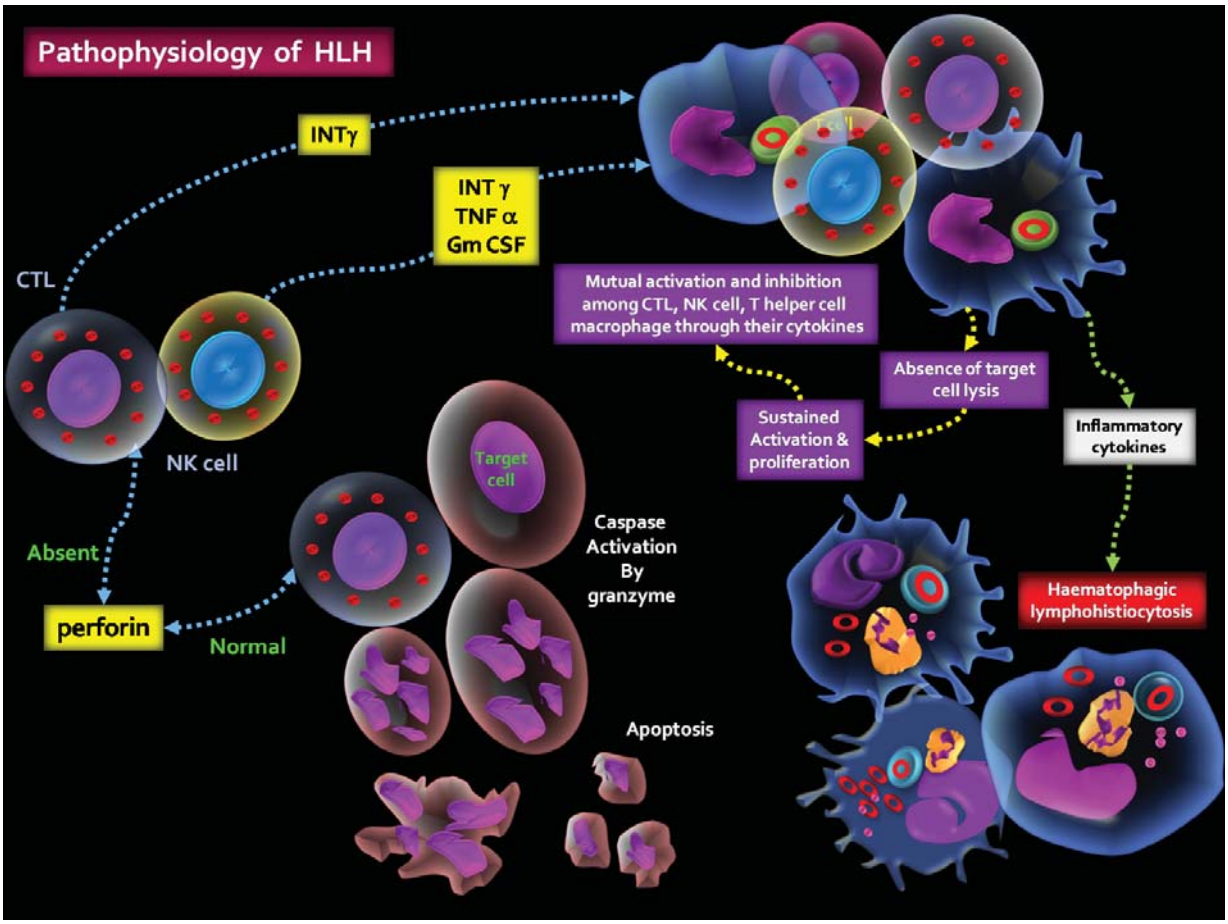


Fig. 7: Depicts the pathophysiology of HLH in patients with perforin defect. Due to defective perforin, NK cell & CTL (Cytotoxic T Lymphocyte) cannot effectively kill virally infected cells resulting in a large amount of secretion of their cytokines like Interferonγ, TNF (Tumor Necrosis Factor)α & GM-CSF (Granulocyte Macrophage Colony Stimulating Factor), which activate Macrophages which then phagocytose all other blood cells. Since the cytokines are produced in a massive amount by Macrophages, T cells & NK cells; hyper stimulation continues and the patient has a cytokine storm with signs & symptoms of HLH. Cytokines are products secreted by cells: TNFα, IL.6, G-CSF & others.

Chronic Granulomatous Disease:

CGD Case 1

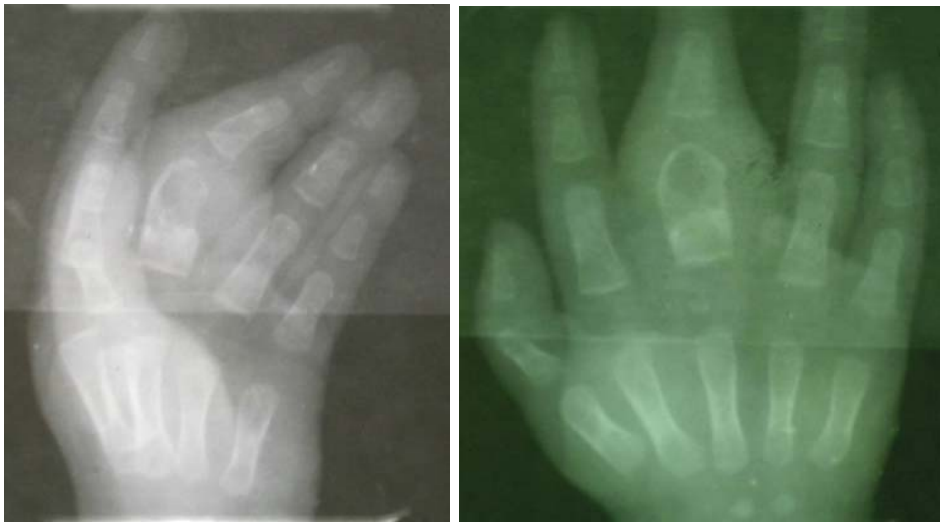
B, a 3 month old boy, presented with a swelling of the right hand middle finger of 1 month duration and fever for 2 days.

Personal history: 1st child of non-related parents, & he received BCG vaccine at birth. Patient had Staphylococcus aureus meningitis at 1 ½ month of age.

On physical examination weight was 2.75 kg (birth wt 2.5 kg), Rt middle finger was swollen (Pic.5), Liver 3 cms & Spleen 3 cms were palpable.

Pic. 5: shows B with Rt hand middle finger proximal phalanx swelling.

His CBC was as follows Hb 7.9 gm / dl, WBC 26,300 / mm^3 – P 68%, L 32%, Platelets 641 x 10^9/L. CRP was increased at 78 & ESR was 90 mm at end of 1 hr. X-rays of bone showed osteolytic lesions consistent with 0steomyelitis of R middle finger (Pic.6). The middle finger contained pus which was drained and the culture grew Enterobacteriaceae (an opportunistic organism).



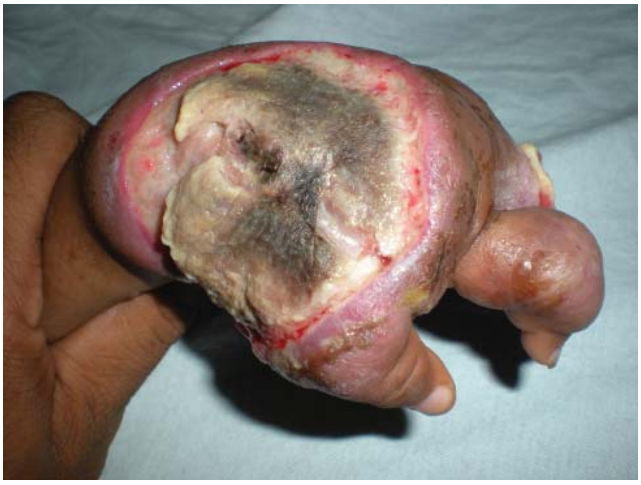
Pic. 6: X ray showing soft tissue enlargement of the middle proximal phalanx & osteolytic lesion suggestive of osteomyelitis.

In view of osteomyelitis of finger & past history of staphylococcal meningitis, the child was worked up for an underlying immune deficiency. NBT was < 1%, hence the diagnosis of Chronic Granulomatous Disease (CGD) was made.

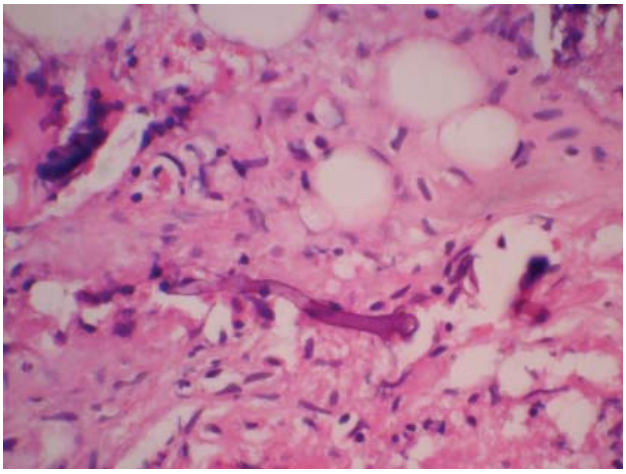
Mother's nasal swab was cultured & grew coagulase - negative Staphylococcus aureus.

Azithromycin was given as prophylaxis for CGD as the patient was allergic to Septran. No anti-fungal agent was recommended at this moment. (discussed below)

CGD Case 2: Cover page case & inside front cover query is related to this case



Pic. 7: 10 mth old child with IV site fungal infection prompting need for work up for PID which confirmed CGD



Pic. 8: Biopsy from the dorsum of wrist showed Basidiobolus fungus; as seen in the photomicrograph.

CGD is a phagocytic disorder. Phagocytic Neutrophils (PMN) commonly ingest & kill bacteria Staphylococcus aureus & fungus Aspergillus sp. The ingestion of an organism and formation of a phagosome is normal but there is a defect in the respiratory burst oxidase (NADPH oxidase) that kills the microorganism, see Fig. 8 with legend. Clinically, the result is recurrent, perhaps life - threatening infections especially observed in (LNs) Lymph Nodes, pneumonia & abscesses. Later, inflammatory granulomas are formed & are responsible for obstruction of gastric or urinary outlets.

In our CGDs, although presently 12 in number, the fungus Aspergillus was found in 50% of cases at some time or the other.

The NADPH oxidase is composed of 5 components located at different chromosomes. In the USA, 60 - 70% is X-linked CGD, due to defect in gp91phox, 30% autosomal recessive due to defect in p47phox and <10% of autosomal recessive CGDs are due to defects in p22phox & p67phox. The 5th component is p40phox. In Muscat, Oman, the p47phox defect was detected in 12/13 patients & only 1/13 was due to gp91phox, probably because of consanguineous marriages in 60%. A dominant negative mutation in Rac 2 can also lead to deregulation of respiratory burst fig. 8.

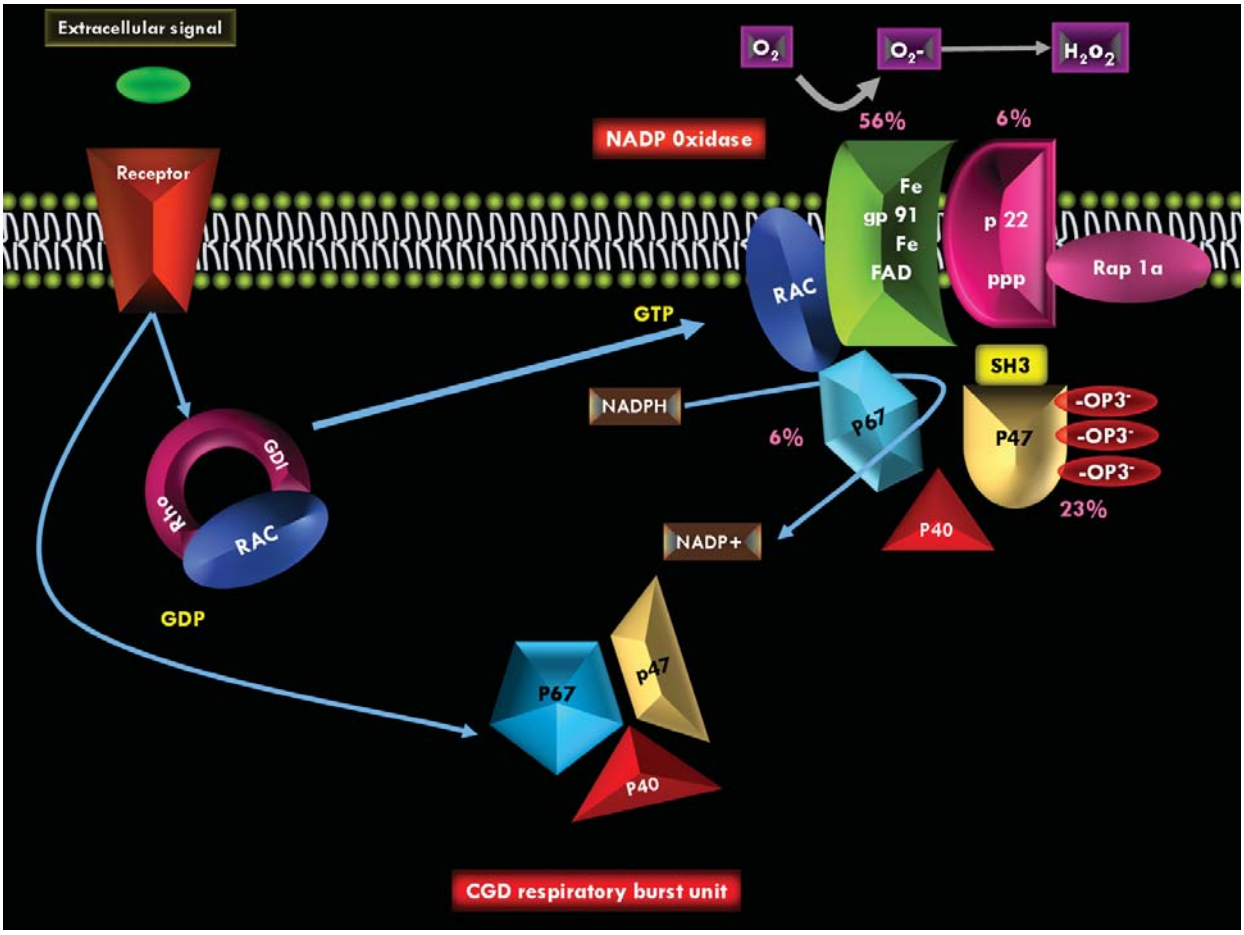


Fig. 8: Respiratory burst in phagocytic cell. The NADPH Oxidase respiratory burst is made up of several components. Some of these are membrane bound like gp91 and p22 while p67, p47 & p40 are Cytosolic components which are brought to the surface upon receiving an appropriate signal. This also results in translocation of Rac & Rap1a to the membrane. This forms the complete NADPH complex resulting in formation of super oxide, HOCL & H_2O_2 necessary for killing of phagocytosed micro organism. Defect in any one of these components can result in clinical phenotype of CGD (Chronic Granulomatous Disease). Patients of CGD are particularly prone to infections by Catalase +ve organisms Staphylococcus aureus, Burkholderia cepacia, Aspergillus, Serratia marcescens, Nocardia & Atypical mycobacteria. Therefore before sending for the study of receptors on Macrophages & T cells (i.e. IL12/23-IFNγ loop defects) to make a diagnosis of IL12Rβ1 we always do an NBT & DHR study to rule out CGD. The cartoon also depicts the relative frequency of these defects in western literature. Inheritance of gp 91 is X linked while p22, p47 & p67 are autosomal recessive.

Pre-natal diagnosis is obtained on fetal blood (after 16-18 wks) by measuring NADPH oxidase activity, or chorionic villi or amniotic fluid samples are taken for DNA analysis. We have done this in 2 cases.

Treatment:

Prophylaxis: to prevent bacterial & fungal infections because the patients can get serious infections from them.

Therapy: Surgical drainage for abscesses in infected LNs. Some patient lack Kell antigens on RBC, hence blood transfusion should be given after testing for the Kell Ag.

For granulomas, corticosteroids; for CGD curative treatment is SCT. Gene replacement therapy is tried in CGD with encouraging results

Staphylococcus aureus abscesses:

VG, a 9 month old male, was admitted for swelling on the left cheek. There was a history of fall from the bed 20 days ago.

Personal history: He is the second child, adopted by the maternal uncle.

Past History: He had a history of right second toe abscess on day 15 of life that required incision & drainage and on culture grew methicillin resistant Staph. aureus (MRSA), He had multiple abscesses all over the body, and a dislocation for which an internal fixation was done.

4 month ago he had a swelling over the Rt. side of chest & X-rays revealed rib osteomyelitis for which a 5th rib resection was required. This event was complicated by Pneumothorax. Culture from the abscess site grew S. aureus & the biopsy of the rib was consistent with chronic non specific inflammation, but no granulomas were seen.

On physical examination child was febrile, weight 9kg, Ht 65cms & B.P. 90/70mm Hg

The Lt. cheek appeared swollen, red & no Lymph nodes were palpable.

Hb was 10.6 gm/dl, WBC 15,200 /mm^3, Poly 46%, Lympho 45%, Monocytes 9% & Plts 180 x 10^9/L.

Serum Immunoglobulins were normal IgG 935 mg/dl, IgM 185 mg/dl, IgA 74 mg/dl, IgE < 17.6 IU/mL

Flow cytometry showed a normal ALC (Absolute Lymphocyte Count) 6992/mm^3 & a normal lymphocyte subsets of CD19, CD3, CD4, CD8, CD16/56 (B, T & NK cells).

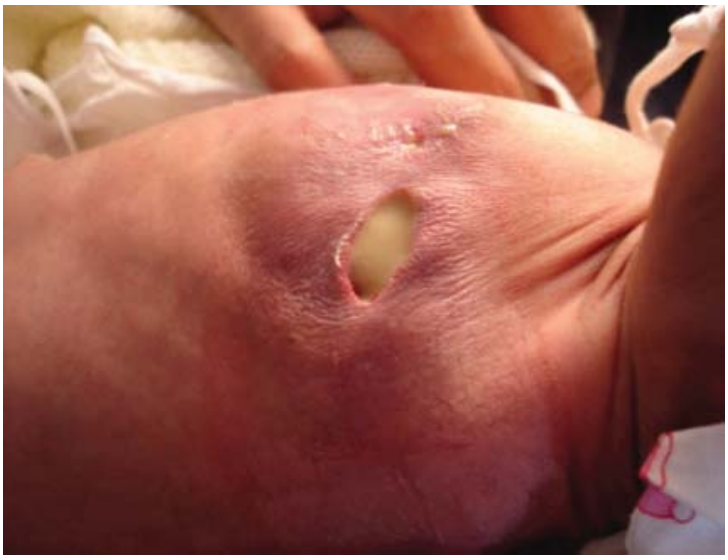
NBT was 98% (normal for CGD).

His viral markers for HIV, HBsAg & HCV were negative.

Gram's stain of pus from the cheek showed presence of Gram +ve cocci in clusters (S. aureus).

Staph abscesses account for 5% of admissions to the surgical wards. Usually, the abscesses are single, can be multiple, uncomplicated & not recurrent, & the causative organism is Staphylococcus aureus, either MSSA or MRSA, (that both respond to antibiotics after I & D). If babies do not respond, we prefer using Linezolid due to increasing risk of community acquired methicillin resistant (MRSA) strains of Staphylococcus aureus. What is surprising & a matter of grave concern is that the age of the onset ! The vast majority are from 0 to 2 months of age, and implies that our community acquired Staph. aureus infections are increasing, & they are more virulent. The concern was also whether he

had an underlying PID. After collecting several cases of abscesses, we realized that most of them did not have a PID. Our present policy is to wait for further PID manifestations before we do an expensive immune work up.



Pic.9: Show a 3 mth old infant with subcutaneous Staphylococcus aureus abscess

Diseases associated with BCG vaccination

BCG vaccine is a live mycobacterial vaccine given to all Indian babies usually on the first or second day of life. Side reactions are usually of little consequence. BCG is given to modify disease from the more virulent mycobacterium tuberculosis in children. However, few children because they are immune compromised may react adversely to the extent that the vaccine may prove fatal. The reasons for any adverse reaction were investigated, & the following were identified.

1. Inherited Mendelian Susceptibility to Mycobacterial Disease (MSMD), probably in > 50 %
2. Severe combined immune deficiency (SCID) leading to fatality in 1/3rd of cases.
3. Very few due to HIV / AIDS.
4. A defect in neutrophils in10% of CGD patients.

All are inherited genetic defects except for AIDS, a viral CD4+ T cell defect.

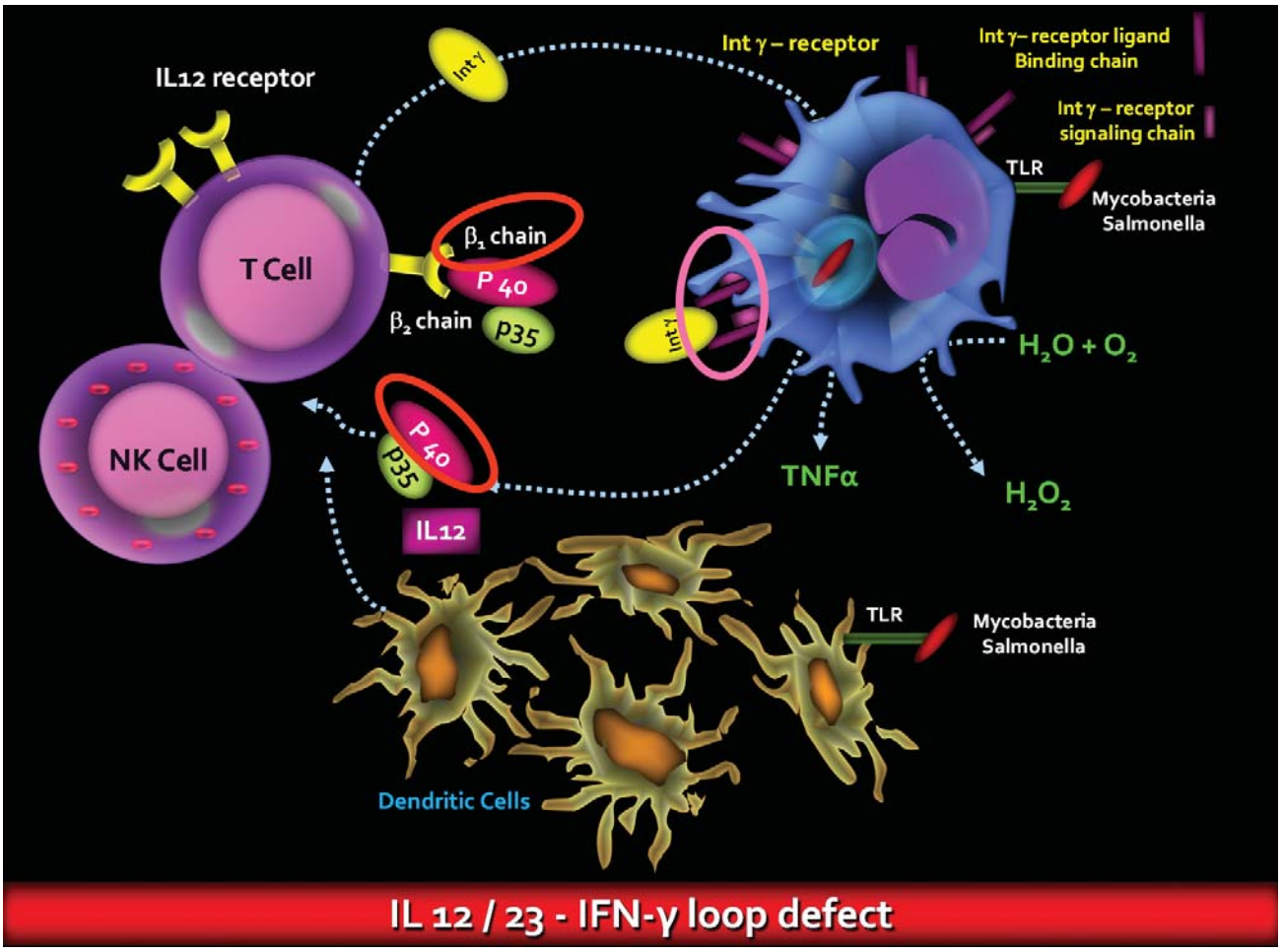


Fig. 9: IL12/23 - Interferon γ loop is important for control of intracellular organisms like Mycobacterium tuberculosis, Atypical Mycobacteria, BCG, Salmonella typhi, non typhoidal Salmonella & Listeria monocytogenes. On ingestion of these intracellular organisms the macrophage secretes IL12. Upon ligation of IL12 to its receptor, T cells & NK cells secrete Interferon Gamma (IFNγ). which binds its receptor on the macrophage and activates a host of genes including the respiratory burst NADPH oxidase which will allow the macrophage to kill the ingested bacteria. Without IFNγ help macrophages fail to kill intracellular bacteria and they can survive for years within the phagosome of macrophages.

MSMD can cause fatal disease, moderate disease, or no disease with BCG vaccination. The BCG mycobacteria Ag enters a macrophage in the body; it is digested, and its Ags are presented on their surface in the groove of MHC class I & II molecules as it is an Ag presenting cell (APC). The other antigen presenting cell (APC) playing an important role is dendritic cells (DCs). APCs on one side and the responders (T cells & NK cells) on the other side and the signals that pass between them IL12/23 and IFNγ (hence called the IL12/23-IFNγ axis) along with STAT 1 and CD40L–CD40, stimulate the immune system to effectively give protection against 2 organisms: tuberculosis & salmonellosis. Fig. 9.

In population studies done in Europe & later global studies over 6 yrs on 1966 subjects, 113 (13%) were identified with MSMD. In 58% complete IL12Rβ1 and IL12p40 gene defects were present, 26% had IFNγR1 complete or partial defect, & 10% had STAT 1 defect. MSMD children are also susceptible to Salmonella infections, intestinal or in half the children, extra intestinal (skeletal & meninges- meningitis or focal brain abscesses).

In 1996, the defect that resulted in BCG disseminated infections was due to mutations in 5 of 6 genes; IL12p40, IL12Rβ1, IFNγR1, IFNγR2, STAT 1 and the 6th is NEMO. These faulty genes are responsible for 13 different phenotypes resulting in susceptibility to mycobacterial diseases called MSMDs, whether the bacterium is the poorly virulent Environmental Mycobacteria (EM) or BCG or the more virulent MTB and the poorly virulent non typhoidal Salmonella or more virulent Salmonella. (see fig. 10)

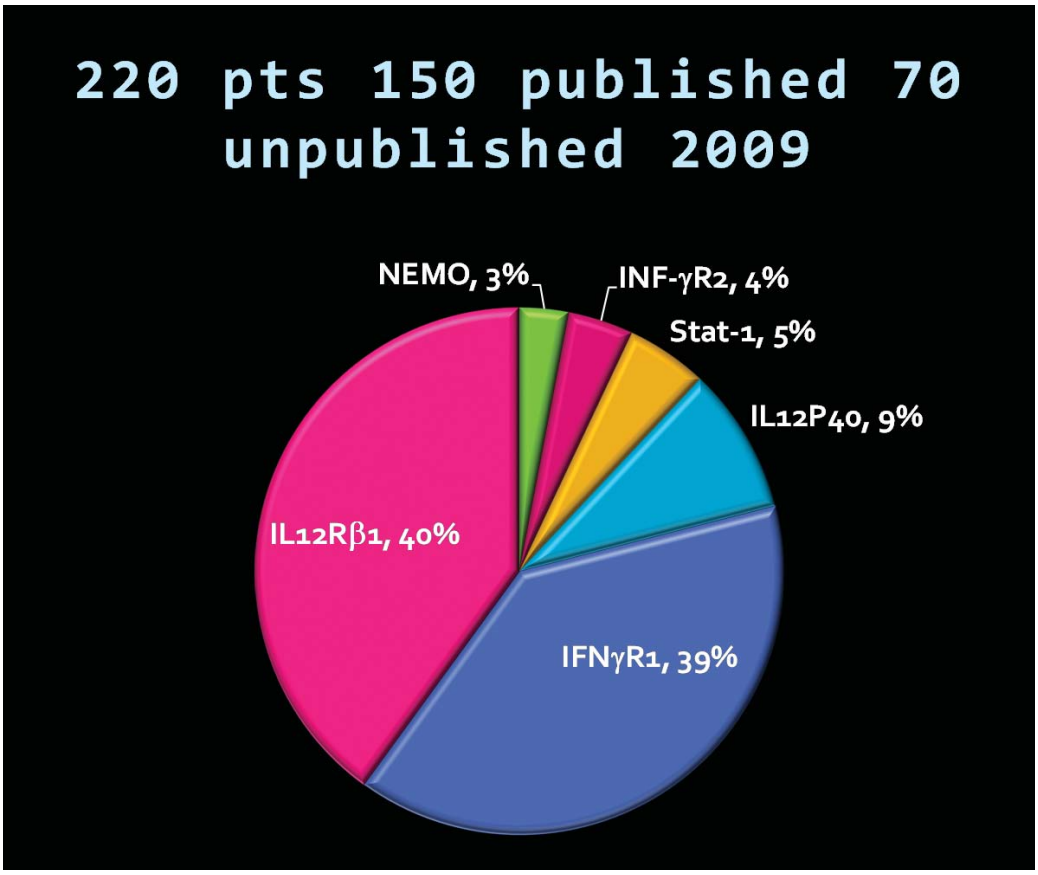


Fig. 10: Show distribution of MSMD in 2009 (JL Casanova personal communication.)

Adapted from Orchid´ee Filipe-Santos et al., Inborn errors of IL-12/23 and IFNγ mediated immunity: molecular, cellular, and clinical features, Seminars in Immunology (2006), doi:10.1016/j.smim.2006.07.010

What is interesting is the reactions caused by any one of the faulty genes can be different. This is beautifully illustrated in a Gujarati family by Dr. J L Casanova from the children's hospital Necker in Paris, France.

3 children of first cousin parents:

1st daughter received BCG vaccine at birth and died at 5 yrs of age of disseminated BCG disease.

Brother was vaccinated also, had no adverse effects and is now living, at 15 yrs of age.

Youngest sister was therefore not vaccinated. Unfortunately being an MSMD candidate, she suffered from 4 episodes of Salmonella enteritis infection between the ages of 2 & 4 years. However she responded well to the antibiotic treatment and has been disease free since. She is currently 9 yrs old. This is an example of IL12 deficiency in which the same gene defect in this family induced a fatal reaction, no reaction, and repeatedly a non virulent Salmonella reaction.

Prediction about the course of MSMDs after BCG vaccination is difficult unless a mutation assay of the involved genes is done (an expensive test) & justified only if the previous sibling died of severe disease after BCG vaccination. All healthy siblings must be closely followed for Mycobacteria and Salmonella infections and investigated if possible.

We submitted blood from BCG vaccinated children who showed reactions and Dr. Casanova has shown to date, that our common defect is IL12Rβ1 defect. Serum IFNγ levels estimation is one test that can be done to distinguish complete IFNγR1 deficiency from the other MSMD mutations. A high serum level of IFNγ with severe BCG / EM (Environmental Mycobacteria) disease suggests complete IFNγR1 or complete IFNγR2, or STAT1 deficiency. Low levels of IFNγ are seen with IL12p40, IL12Rβ1, or partial IFNγR1 or R2 deficiency Fig 11.

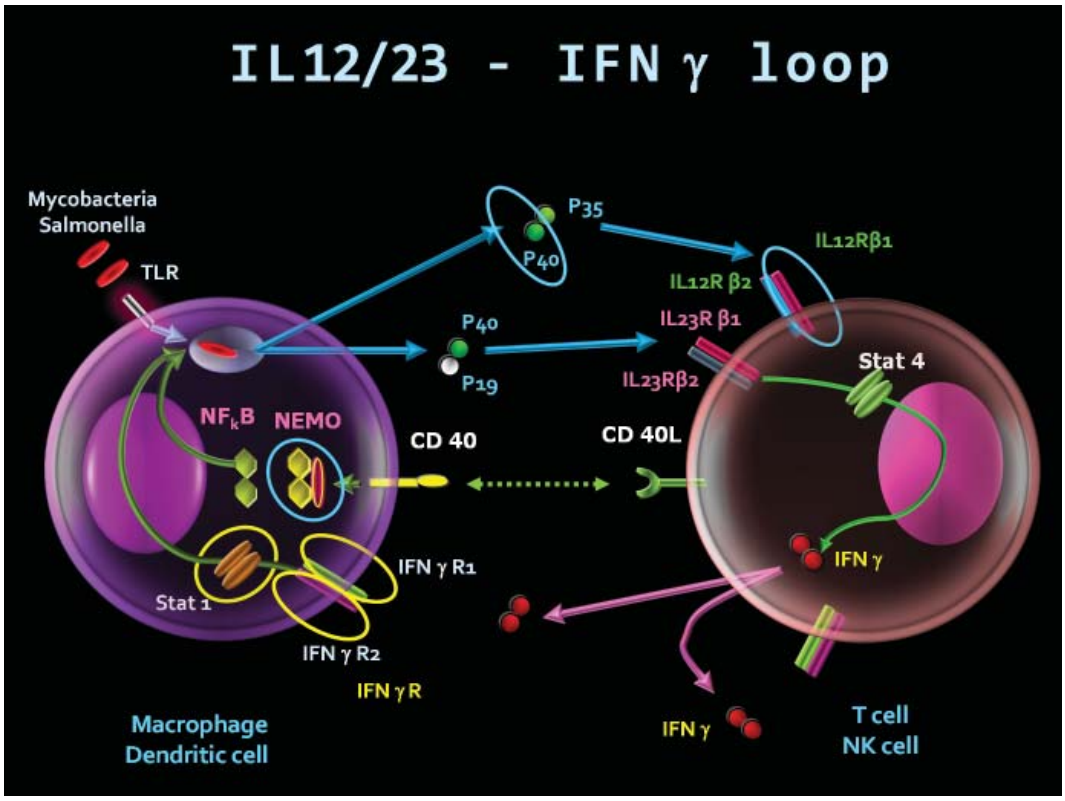
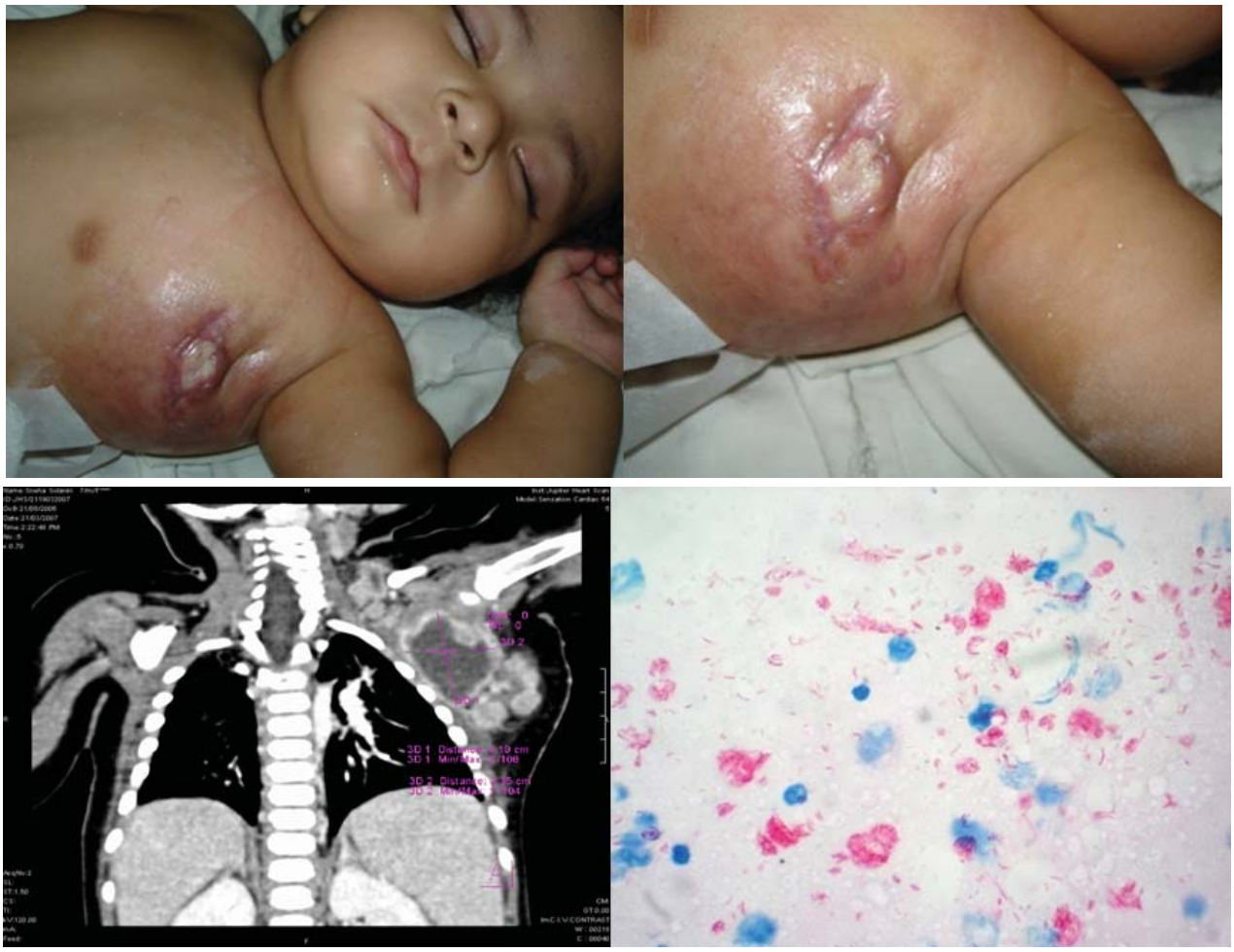


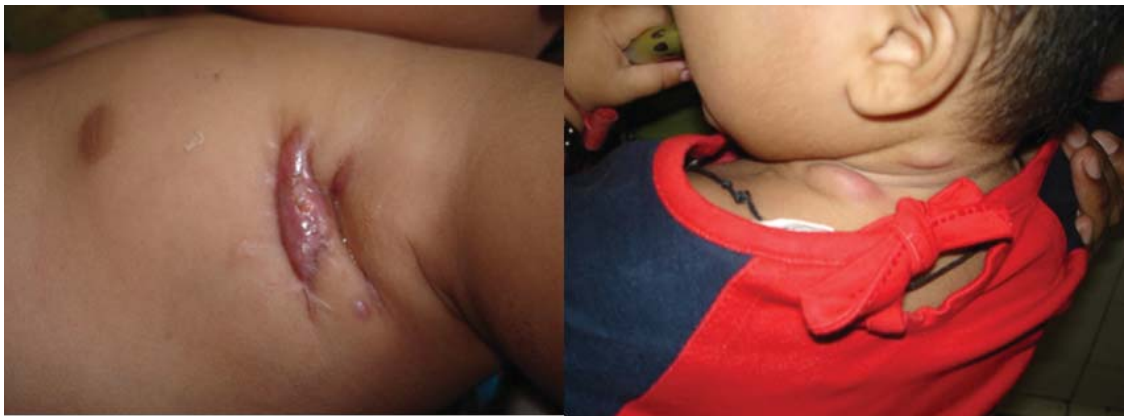
Fig. 11: Since IL12Rγ1 is shared by IL12R & IL23R it is called IL12/23-IFNγ loop. This figure highlights defects marked by Blue circles which result in defective IFNγ production while defects marked by yellow circle depicts defects resulting in poor response to IFNγ.

Look for granulomas in various organs. Granulomas are formed because of chronic stimulation by mycobacteria and by IFNγ. Granulomas are composed of Mϕs, T lymphocytes & epithelioid cells so that the infection can be controlled & does not spread. Half the patients with disseminated BCG have tuberculoid Type 1 well defined granulomas and surrounding fibrosis which contain few AFB, and lepromatous type 2 like lesions in the remaining patients with poorly formed granulomas containing large numbers of AFB and the prognosis is bad.

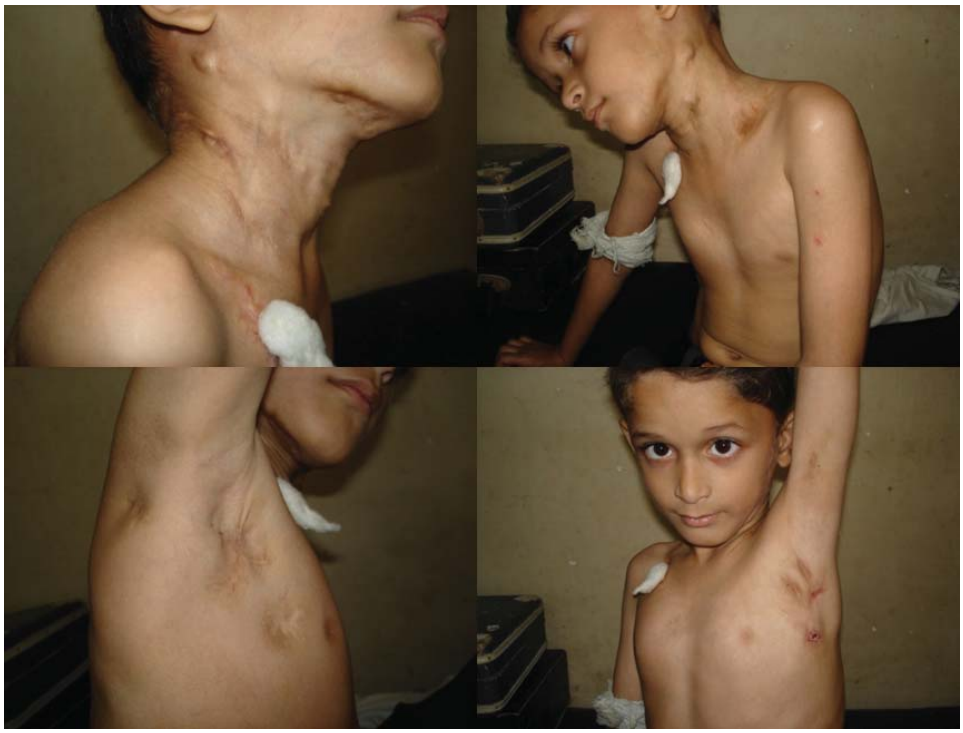
Some of our clinical cases with BCG vaccination, EM in our babies are shown in Pic 10, 11, 12, 13, 14, 15 & 16:



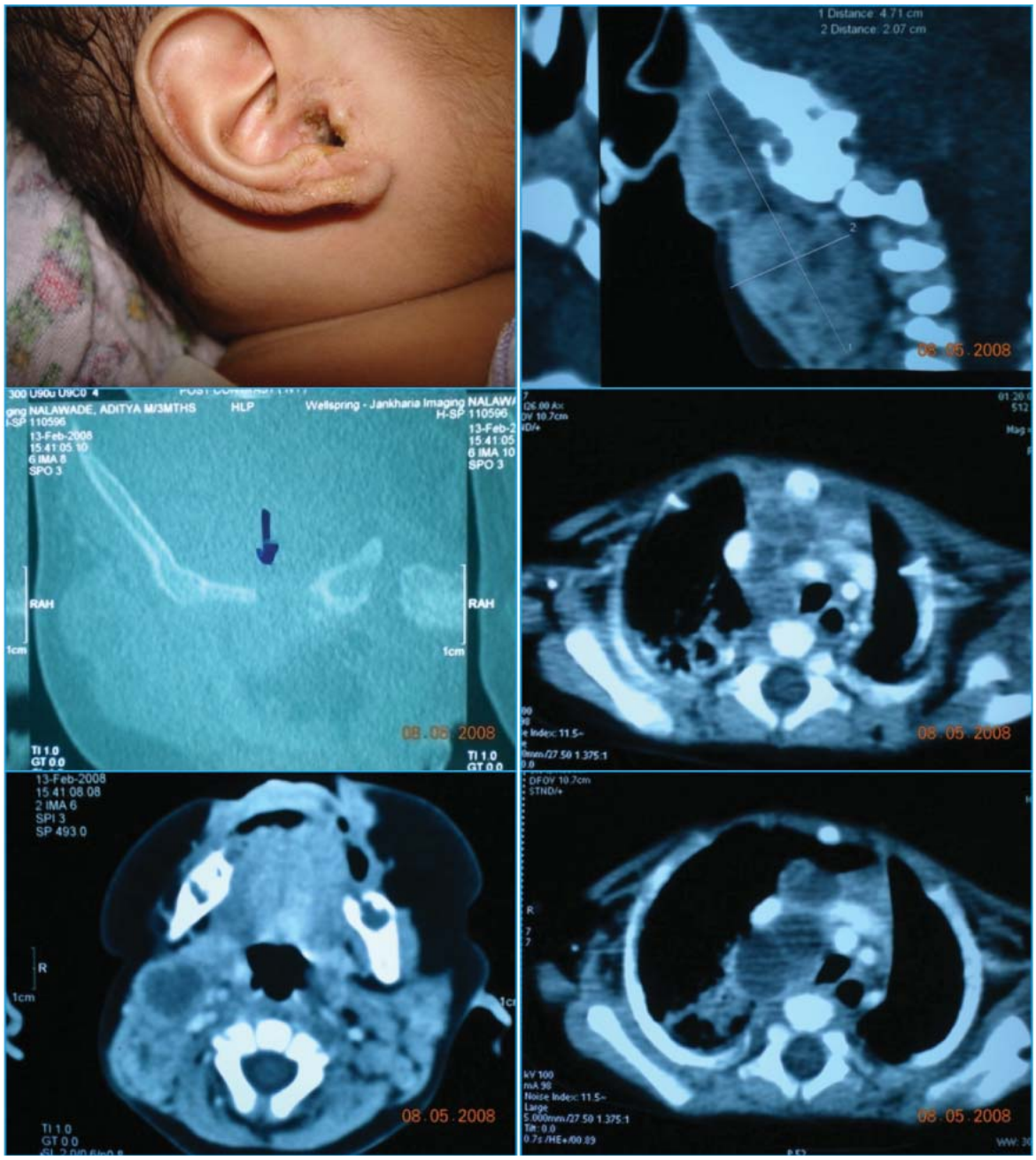
Pic. 10: Picture & image of a child with disseminated BCGosis. Note the massive left axillary necrotic LN; also note that this baby has no FTT. Work up of this child revealed IL12Rβ1 defect.



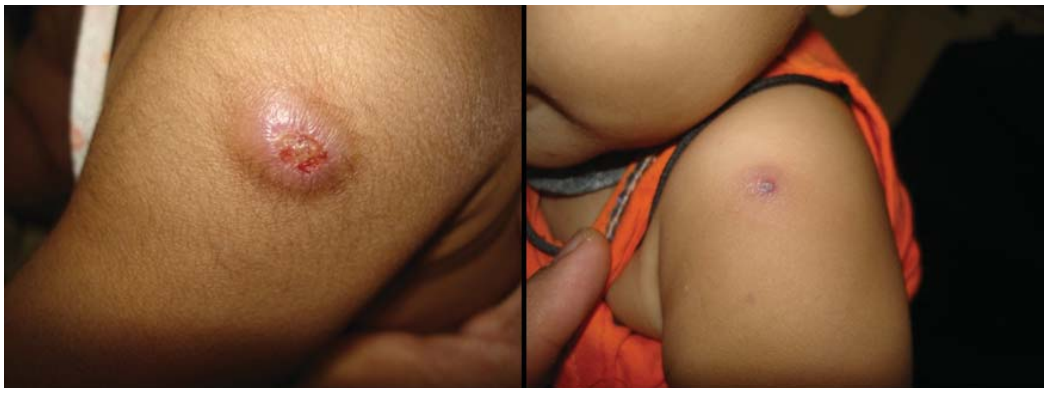
Pic: 11: Follow up images of the same child with healing of the Left axillary necrotic node but recurrence of cold abscess at another site Lt supraclavicuar & cervical LN.



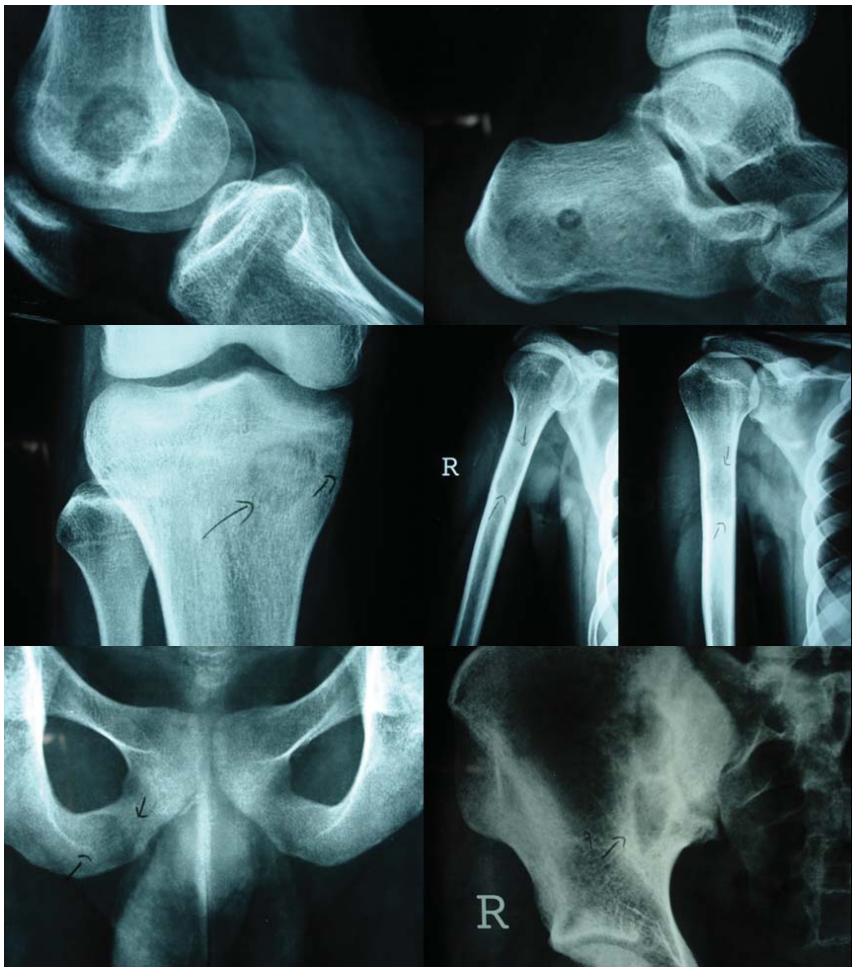
Pic. 12: Show a 10 yr old boy who first presented at 6 mths with BCG adenitis & recurrent suppurative lesions partially responding to antibiotics. He was referred to us from TATA hospital as a case of CGD who could not be transplanted as he did not have a matched donor. During follow up with us we found that his CGD work up at NIIH was normal. He kept having recurrent problem with Lymphadenopathy and vasculitic skin lesions and impetigo. We biopsied his Lymphnode and besides sending it for histopathology, cultured it and the organism isolated was non typhi salmonella which gave us the clue that this is a case of IL2/23-IFNγ loop defect most likely IL12Rβ1 or IL12p40 defect. Always culture any tissue biopsied as isolating an organism can provide an important clue to the type of PID.



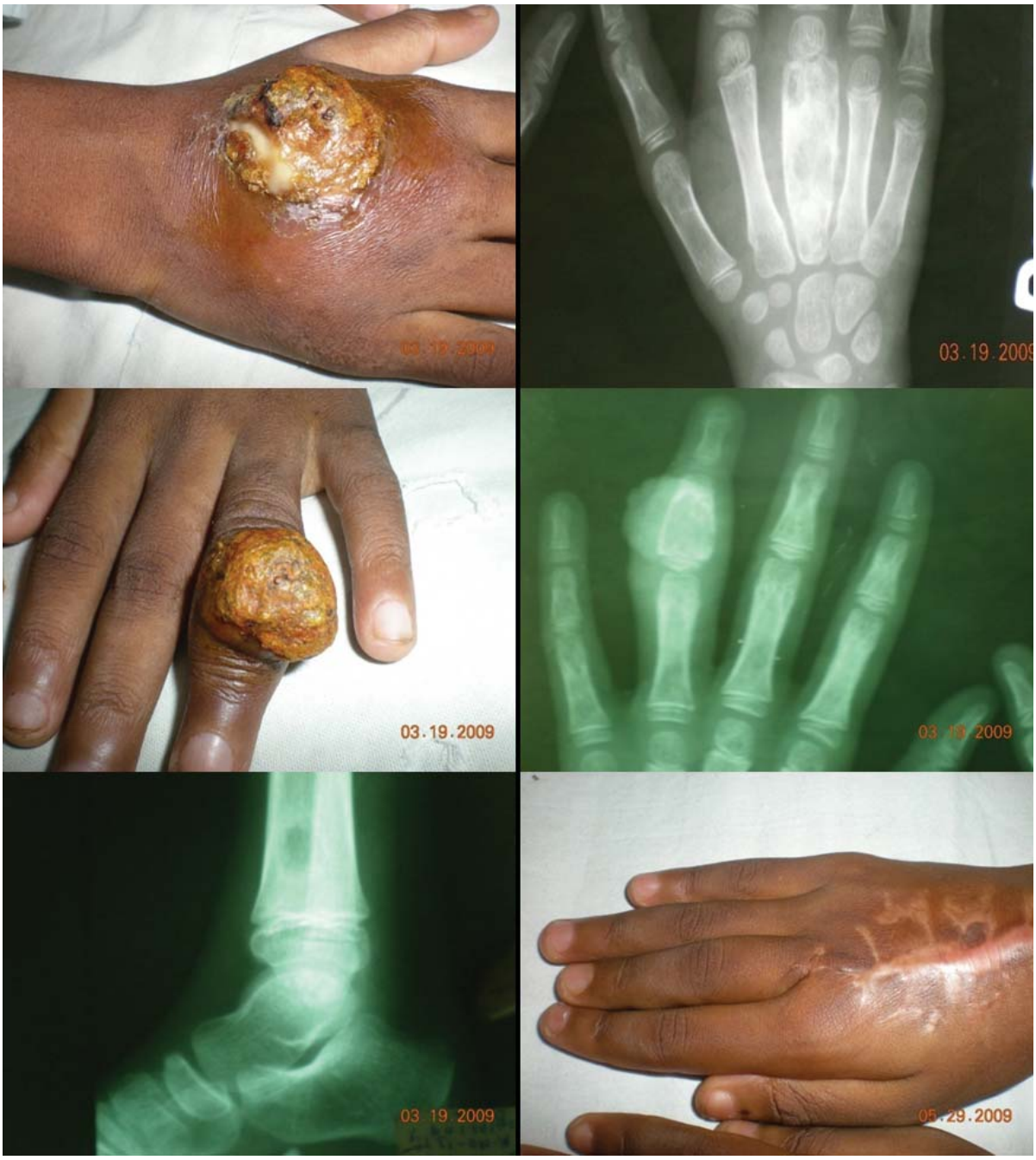
Pic. 13: AD presented to us with ear discharge and was diagnosed as a case of middle ear mass (? Rhabdomyosarcoma). He was operated and the histology was suggestive of Koch with a granulomatous lesion with few AFB seen on ZN stain. He also developed cervical, occipital, mediastinal necrotic lymphadenopathy along with HSmegaly. A diagnosis of disseminated BCGosis was made and since his Lymphocyte subset was normal we suspected a defect in IL12/23-IFNγ loop defect. Since the granuloma was well formed with few AFB & osteomyelitis involving external auditory canal the possibility of partial defect of IFNγR1 or IFNγR2 is likely.



Pic. 14: Shows BCG scar which should alert a clinician to possibility of PID, either T cell defects or IL12/23-IFNγ loop defect.



Pic. 15: Shows a 23 yr old boy with multifocal bone tuberculosis proven on histology. PD-IFNγR1 (Partial Dominat IFNγR1) defect should be suspected as almost 80% of these patients present with multifocal bone tuberculosis.



Pic. 16: Shows another example of multifocal bone tuberculosis possibly secondary to PD-IFNγR1 defect

Table 2: Location of BCG disease:

C = complete; P = Partial; AR = Autosomal Recessive; AD = Autosomal Dominant;

STAT = Signal Transducer & Activator of Transcription; RES* = Reticuloendothelial System

	IFNγR1	AR-IFNγR1	AR-IFNγR2	AD-IFNγR1	C IL2-P40	C IL-12Rβ1	STAT 1
RES*	+	+	+	+	+	+	+
BONE	+			+			
CNS	+						
GI							
RS	–	–	–	+	–		
SKIN	+	+	+	+	+		

BCG VACCINATION:

Disease location in BCG in various MSMD defects are shown in Table 2 & 3

Table 3:

If vaccinated	BCG disease	EM	SALMONELLA
In IL-12Rβ1 & 1L12p40 defects	In 57%	No BCG But EM+	+
In IFNγR1 defect Recessive complete	+ in 100%		+ in 14%
In IFNγR1 defect Dominant partial	+in 73%		+in 5%
In STAT 1 Complete	+ disseminated	+	–
In STAT 1 Partial	+ disseminated		

Part III

B cell defects

Adaptive Immunity:

By B lymphocytes (Humoral Immunity)

By T Lymphocytes (Cellular immunity)

Combination (Cellular & Humoral Immunity)

Humoral Immunity:

B cells develop in the bone marrow (BM) and on becoming mature leave the BM, go to lymph nodes (LNs) & when they encounter an Ag (antigen) that is recognized by their BCR (B cell Receptor) they form germinal centres (G.C.) in follicular LNs, and then to become memory B cells for a quick response when the same Ag is encountered, or proceed to becoming a plasma cell (PC) which make antibodies (Ab) against the pathogen which is most commonly bacteria.

In PIDs, the fault lies in the BM and today we can point at which stage of B cell development the fault occurs by markers e.g. Btk, CD 19.

CD 19 & CD 20 are B cell markers; Ig has 2 chains: [Heavy μ, α1, α2, γ1, γ2, γ3, γ4, δ, ε1 & ε 2 + Light (κ + λ)]

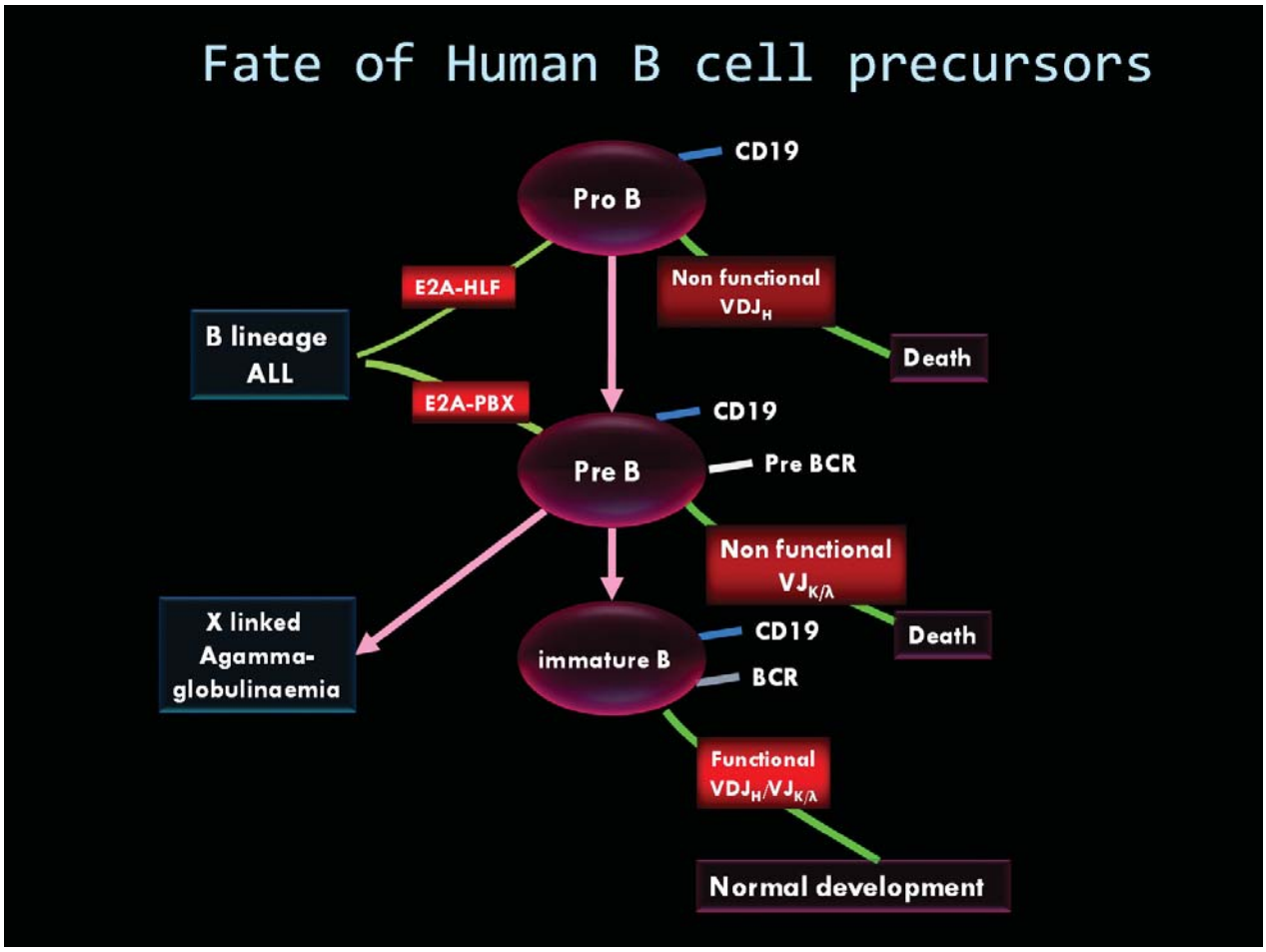


Fig. 12: show early stages of B cell development within the BM from CLP (Common Lymphoid Progenitor) to more committed ProB cells to PreB cells to immature B cells. During this process the BCR is formed by recombination of various segment of Immunoglobulin gene. Any defect in this process can result in either XLA (X linked Agammaglobulinemia) or B lineage ALL. A successful VDJ recombination allows B cell to proceed to mature B cell stage. Unsuccessful VDJ recombination result in apoptosis.

BCR= B cell receptor; VDJ = components of BCR; Variable, Diversity & Joining.

XLA (X linked Agammaglobulinemia)

Defects in B cell lineage during development leads to Antibody Deficiency Syndrome (ADS). Agammaglobulinemia is the commonest & severest of all ADS with a significant reduction in serum Igs; serum IgG< 200 mg/dl, IgM & IgA < 20 mg/dl; known as XLA (X linked Agammaglobulinemia)

The block in development is between Pro-B and Pre-B, because of a lack in the enzyme Btk (Bruton Tyrosine kinase) Fig. (12, 13).

Children with agammaglobulinaemia are susceptible to infections with the following organisms, Haemophilus influenza, Streptococcus pneumonia, Pseudomonas aeruginosa, Staph. aureus to a lesser extent, Enterovirus (e.g Echo virus & Polio virus) & Giardia lamblia.

Children with ADS are not susceptible to fungi, coliform organisms, TB, BCG, and many viruses like Measles, Chicken-pox, Rubella, Vaccinia;

Do not give live polio vaccine.

Onset of symptoms begin after transplacentally transmitted mother's Abs (IgGs) to the newborn disappear i.e. between 6 to 12 months of age. However, a non PID baby is capable of making IgM in response to any infection and if the IgM is absent, it suggests that the baby has a block in B cell development, resulting in a PID called Bruton's agammaglobulinaemia, an X linked PID, hence called XLA (X Linked Agammaglobulinaemia).

Inheritance: Since the inheritance is X-linked, elicit a history of repeated infections in males from the mother's side. (Females are affected only if the mutations affect the invariant λ light chain or μ heavy chain which are components of the Pre-B cell receptor) fig. 13

Signs & Symptoms are largely respiratory (sino pulmonary) & / or Gastro-intestinal (Diarrhoea, Giardia lamblia), CNS; (Enteroviruses induced chronic meningoencephalitis), Skin & muscle (Dermatomyositis with a red rash & weakness)

Case study: VH, 5½ years old boy presented with symptoms of intermittent fever since 10 months of age.

Past History:

1. Malaria 2 episodes at 1½ yrs and 3¼ yrs. of age.
2. Lungs and pleural involvement with pneumonia & empyema: between 3 to 5½ yrs of age
3. Urinary tract infection at 3 yrs. of age (pyuria) & hematuria at 5 yrs.
4. Left knee joint swelling at 3 yrs., tapped; synovial biopsy showed chronic villous synovitis
5. Otitis media and persistent ear discharge between 3 to 5½ yrs of age.
6. Wt 14kg constant in past 3 years suggesting FTT (Failure To Thrive)
7. Serum Immunoglobulin levels were markedly decreased with serum IgG < 200 mg/dl, IgM < 20 mg/dl & IgA < 20 mg/dl.

   The diagnosis was Agammaglobulinaemia XLA.

Known problems in XLA:

(1) Mono or Oligo articular involvement of large joints can occur; at times they are sterile due to, in many cases, a non-pathogenic commensal strain of Mycoplasma, or infection with an Enterovirus. (Pic.17)

(2) Recurrent pyogenic infections (Pneumococcus, Staphylococcus, Haemophilus, Streptococcus & Pseudomonas aeruginosa); Otitis media; Pneumonia are common and mainly due to Hemophilus and S. pneumonia, less frequently due to S. aureus and S. pyogenes.

(3) Beware of:

- Chronic meningoencephalitis from Echovirus (Enterovirus), and
- Dermatomyositis like condition due to Enterovirus.

Autosomal Recessive agammaglobulinemia due to mutation in pre BCR Fig. 12, 13

Therefore the block is at Pro-B to Pre-B and with identical signs and symptoms of XLA. The frequency of Agammaglobulinemia is much less in females compared to XLA in boys. Onset of disease is earlier and severe complications are more common.

Case of XLA in a female: 5yr female presented with following salient features.

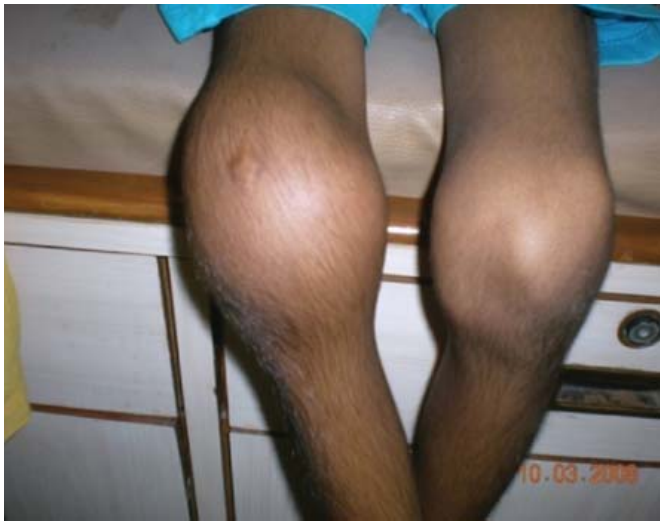
(1) History of repeated LRTI since 9 months of age.

(2) CNS: Chronic Meningoencephalitis (CME) was suspected because she was not interacting with the mother; having purposeless movements; inappropriate smiles and laughter, no speech & neuro regression. CT scan showed cerebral atrophy with dilatation of the ventricles.

(3) Dermatomyositis: skin & muscle involvement were present with dry, scaly skin & icthyosis & progressive muscle weakness.

(4) She also had ankle contractures and a claw hand.

(5) IgG, IgM & IgA were all low and the B cells CD19 & CD 20 were absent.



Pic 17: Child with bilateral knee joint swelling which was being treated as RA with MTX for yrs before being diagnosed as XLA. Jt swelling is classically seen with Ureaplasma urealyticum infection.

Common Variable Immune Deficiency

- CVID is a primary antibody production failure.
- The onset is delayed, i.e. usually after 1½ years of age
- Inheritance is not known in the majority, <10% are inherited.
- **Diagnosis:**

  (1) Serum IgG < Normal with at least one or other serum Ig isotype (usually IgA) < 5th percentile of normal.

  (2) Exclusion of an underlying cause e.g. B cell failure, AID (Activation Induced Cytidine Deaminase) / UNG (Uracil DNA glycosylase) deficiency

  (3) Distinct clinical or laboratory features.

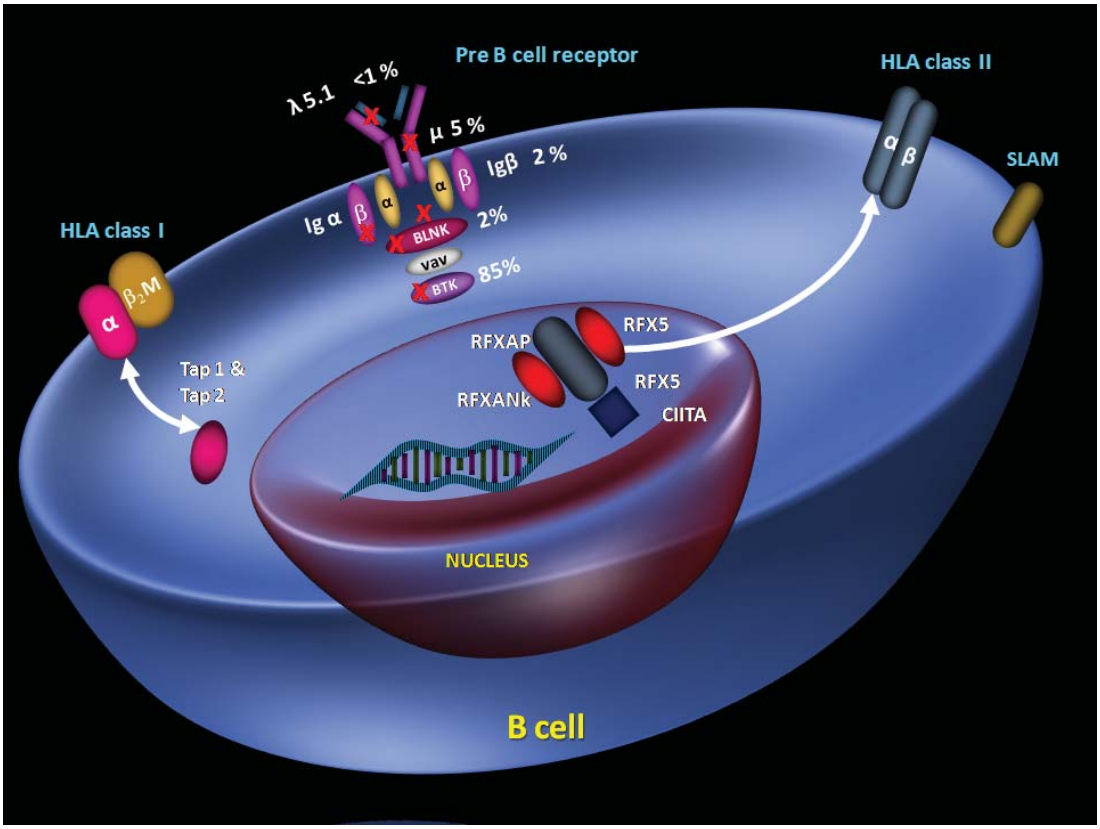


Fig. 13: Shows a combination of BCR - Pre - B stage. Pre - BCR - Between Pro - B & Pre - B stage. Fig. shows B cell receptor (BCR) with its associated protein Igα & Igβ along with downstream adaptor signalling proteins BLNK, Vav & Btk. BCR is an IgM immunoglobulin molecule attached to the B cell surface. The commonest cause of agammaglobulinaemia is Btk deficiency seen in 85% of cases while faulty Pre-BCR due to a μ heavy chain defect is seen in 5 % of cases. invariant lambda light chain defect in < 1 % of cases. and Igα & Igβ defect and BLNK deficiency in 2% of cases each.

- Clinical phenotyping in 334 patients from 7 European centers were analysed, the patients were >4 years of age, with no family history of infections in males, and an absence of previous opportunistic infections.
- Most common age of onset of symptoms was in the 20s but this does NOT exclude infants.

There are 5 clinical categories:

The 5 clinical categories are

(1) No complications

(2) Autoimmunity

(3) Polyclonal lymphocytic infiltration

(4) Enteropathy

(5) Lymphoid malignancy

(83% of patient's show only one clinical phenotype)

Polyclonal lymphocytic infiltrations have a 5- fold increased risk of developing lymphoid malignancies that correlate with IgM at diagnosis.

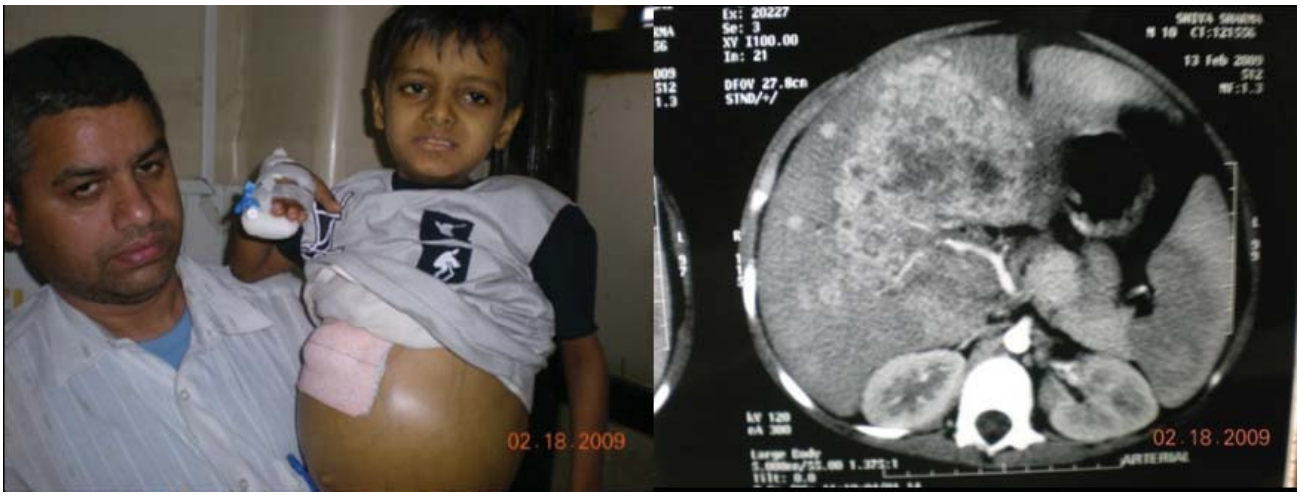
SS, a 10½ yr male, was referred to BJWHC for fever, progressive abdominal distension, and jaundice.

In 7/2008, age 10 yrs he was investigated for recurrent fever, cough, convulsion & jaundice. Work-up revealed an IgG 416 mg/dL (normal 700-1600 mg/dl); IgM 46.1 mg/dL (N=47-240 mg/dl); IgA 28.8 mg/dL (N=21-828 mg/dl) confirming the diagnosis of hypogammaglobulinemia; CVID.

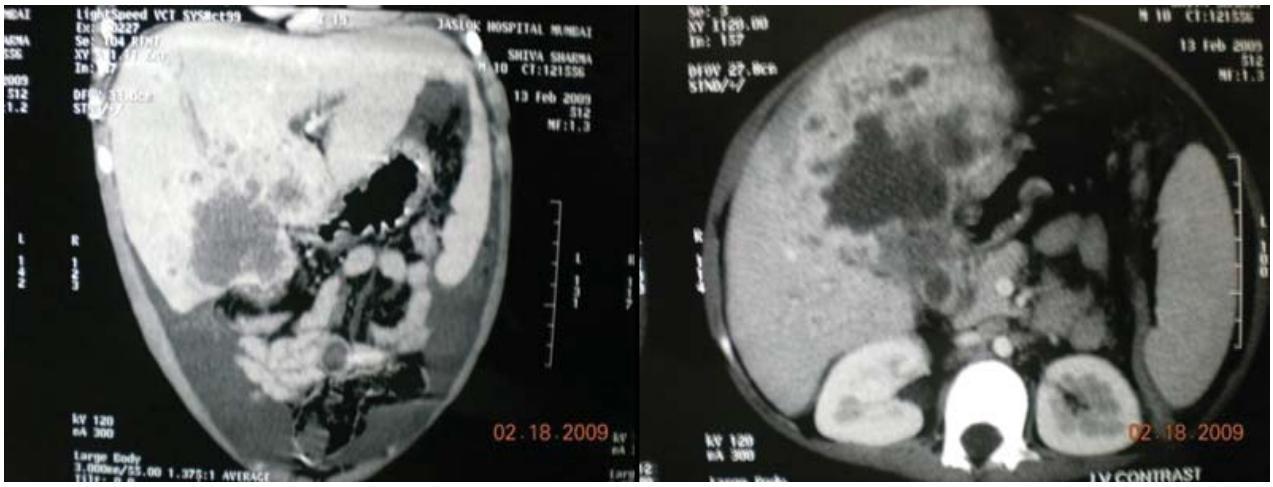
On physical examination, he had a gross hepatosplenomegaly, a palpable mass in liver, ascitis and generalized lymphadenopathy. Abdominal MRI and CT scan is shown in Pic 18 & 19. A CT guided biopsy of the mass in liver was carried out. Ascitic tap showed malignant cells of high grade non-hematolymphoid type. His CEA levels were markedly increased with levels of > 600 (N < 0.33 ng/ml). CEA is increased in Wilms tumor, Hepatoblastoma, Hepatocellular carcinoma, Stage IV Neuroblastoma with hepatic involvement, Germinal cell tumor, Pulmonary blastomas and Retinoblastoma.

LSSA: ALC, CD 19 (B cells), CD3 (T cells) & CD8 (Cytotoxic T cells) were elevated. CD 4 (Helper T cell) & NK cells were normal while CD 8 was 2 times normal.

Significant correlation exists between lymphoid malignancies & IgM > 50 mg/dl



Pic. 18: clinical picture of the patient; with anaemia, ascitis & hepatic coagulopathy



Pic. 19: CT image reveal a large mass in the Rt lobe of liver with areas of necrosis. Patient was diagnosed as a case of CVID and had a CEA > 600 suggesting possibility of a metastatic adenocarcinoma with primary most likely from stomach at the age of 10 ½ yrs.

Table 4: Clinical manifestations & infecting organisms in CVID

Manifestations	CVID	Common Pathogens
Sinusitis	+ + +	Haemophilus influenzae
	+ + +	S. pneumonia
	+ +	Moraxella catarrhalis
Pneumonia	+ + +	H. influenzae
	+ + +	S. pneumonia
Bronchiectasis	+ +	
G.I. infection	+	Giardia lamblia
		Campylobacter jejuni
Splenomegaly	+	
Lymphadenopathy	+	
Conjunctivitis	+	
Meningitis	+	Echo virus
Viral infection	+	HCV, VZV
Cancer Risk	+	
Autoimmunity	+	
Hemolytic Anemia	(+)	
Thrombocytopenia	(+)	
GU infection	(+)	Ureaplasma urealyticum

In CVID, patients with chronic occult infections (e.g. H.pylori, CMV, HHV 8) are predisposed to develop lymphomas (NHL), due to immune deregulation.

T-lymphocyte defects

Children with T cell defects get Infections by

1) Intracellular organisms (Mycobacteria spp)
2) Opportunistic organisms (Predominantly Pneumocystis jiroveci, Cryptococcus & Candida).
3) Disseminated viral infections (CMV, EBV, Adenovirus disseminated Varicella, RSV, Enteric & Herpes)

Consequences of T cell Deficiency:

- Presentation is usually within 6 mths of age
- Chronic infections; especially associated with constant stooling. However persistent infection usually occur with viruses (CMV, EBV, Adeno, Enteric, Varicella, Herpes & RSV), fungii (Candida), and opportunistic organisms, often Cryptosporidium with diarrhoea.

- Fatal GVHD if blood transfusion is given without 3000 cGy irradiation. Lymphocytes in blood transfusion remain viable for up to 3 weeks and can cause Transfusion associated GVHD.
- Maternal lymphocytes persists in 40 %(22/58) of SCID (Severe Combined Immunedeficiency) infants regardless of the SCID genotype. These persistent maternal lymphocytes may cause GVHD (eczema, splenomegaly, eosinophilia and occasionally lymphocytosis with a normal ALC which is misleading).
- Fatal or disseminated reactions if vaccinated with live virus (eg. MMR) or BCG.
- Pay attention to ALC; an ALC <1500/cumm and serum IgGs <150 mg/dl in an infant spells SCID (Severe Combined Immunedeficiency).
- Malignancies: Out of 500 PID tumors, leukemia (12%), lymphoma (73.8%) - (Hodgkin lymphoma 9.5%), gastric sarcoma (2.4%), others (2.4%) were recorded.
- Autoimmunity: Treg defect (Cd4+, Cd25+, FOXP3+), NK & NKT cell defects.

S.G.T: 4½ months boy was admitted on 13.07.2006, for intermittent fever over 1 month, a dry cough since 3 month of age, multiple skin nodules, intermittent oral thrush since 3 months of age, perianal excoriation and diarrhea Pic (19). Child was a FTND with birth weight of 3.5 kg. Present wt is 6.3 kg, Ht is < 5th percentile. On physical examination child was febrile with bulging anterior fontanelle, edematous fingers, gangrenous Rt. toe & a perianal ulcer with red rash over buttocks and legs. Liver was 4.5 cms enlarged. Rt. para cardiac patchy pneumonitis was present on Chest Xray. Na+ was 117 mEq/L, K+ 4.4 mEq/L, Alb 2.4 gm/dl, SGOT 458 IU/L, SGPT 700 IU/L and Blood ammonia was 140 mcg/dl. CSF showed 350 cells (75%.polys, 25%.lymphocytes), Proteins 135 mg/dl, Sugar 10 mg/dl. WBC was 9,900/mm^3 with Poly 88%, Lympho 6%, Monocyte 4% and Eosinophils 2% and a low ALC of 594 / mm^3.

Blood and CSF culture grew Pseudomonas aeruginosa. His D-Dimer was +, HIV was negative, MT 7 mm & Malarial Parasites were absent. Endotracheal secretions grew Candida.

His immune tests revealed a T-, B+, NK+ pattern and an ALC of 594 / mm^3; Igs were very low.

The history is compatible with Severe Combined Immune Deficiency (SCID) with extremely low B & T cell function. He presented before 6 mths of age; he had no tonsils; a rash, persistent respiratory signs, diarrhoea, Candida sepsis & meningitis.

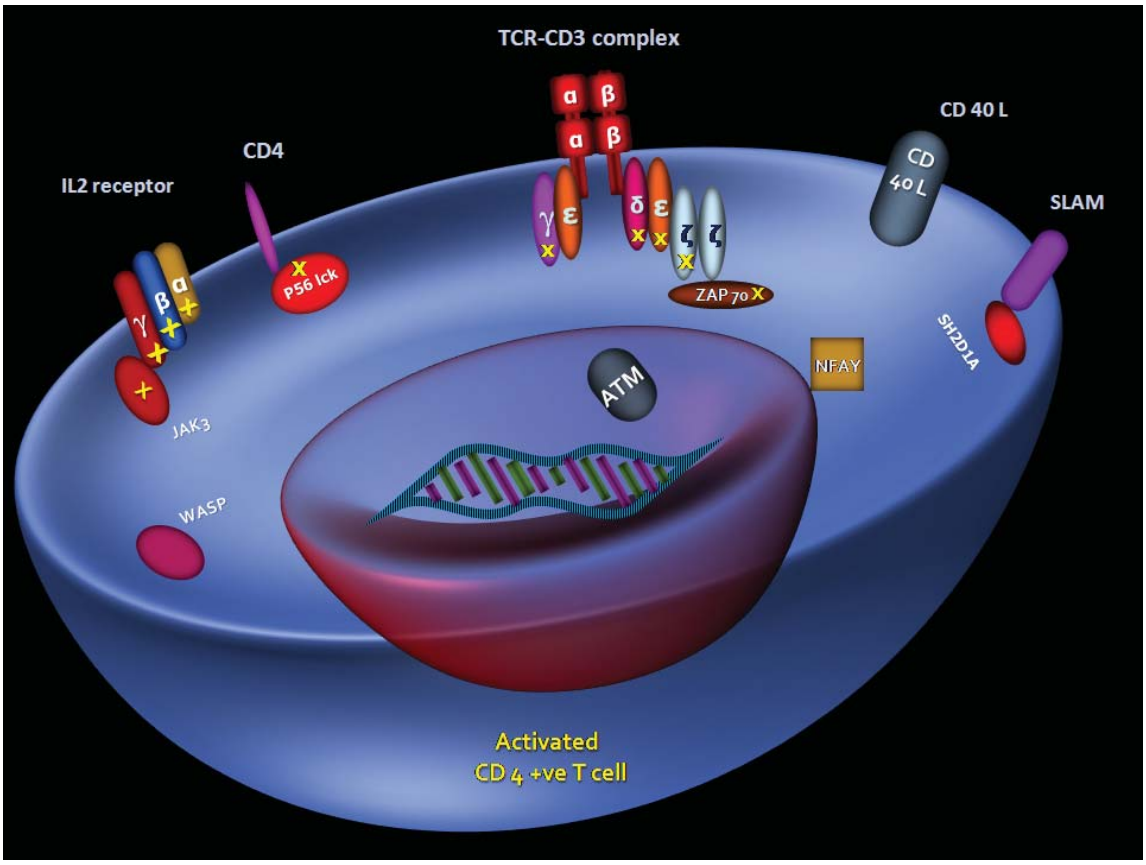


Fig 14: shows various molecular defects which can result in clinical phenotype of SCID. The commonest cause for SCID is due to defect in γ chain of IL.2 receptor or its adaptor protein JAK3 defect. Both have a similar clinical phenotype. IL2 receptor is a hetrotrimer of α, β & γ chain. Defects of IL2R α chain & β chain also result in immunodeficiency. Since TCR (T cell Receptor) does not have intra cytoplasmic extension it is always associated with CD3 molecules important for intra cytoplasmic signalling on engagement of TCR by antigen. CD3 is made up of several protein chains γ, δ, ε, ζ which together form the CD3 molecule. Defects in chains of CD 3 molecules are described and result in SCID. Other adaptor proteins like P56 lck which associates with CD4 molecule, ZAP 70 which associate with ζ chain of CD3 molecule result in distinctive phenotypes of SCID.

Table 5: T cell defects are classified according to block in T-cell differentiation.

T- B- NK-	T- B- NK+	T- B+ NK+	T- B+ NK-	T+ B+ NK+
Reticular dysgenesis	RAG 1 & 2	1L.7Rα	X-SCID(γc)	P56 lck def (CD4↓)
ADA	ARTEMIS	CD3δ	JAK 3 deficiency	ZAP70 def. (CD8↓)
	OMENN's	CD3γ	PNP def	HLA DR (HLA Class II)
	NAVAJO	CD3ε	T^- B^+ NK^{low} CD45 def	IL2R, IL2
			DiGeorge	Cartilage hair hypoplasia (T low, B variable NK may be low)

Molecular defects resulting in Severe Combined Immune deficiency (SCID) is shown in Table 5.

T cell defects or combined T+B or SCID variants all have a block in T-cell differentiation and can variably be associated with defective differentiation of other blood cells see, fig 14.

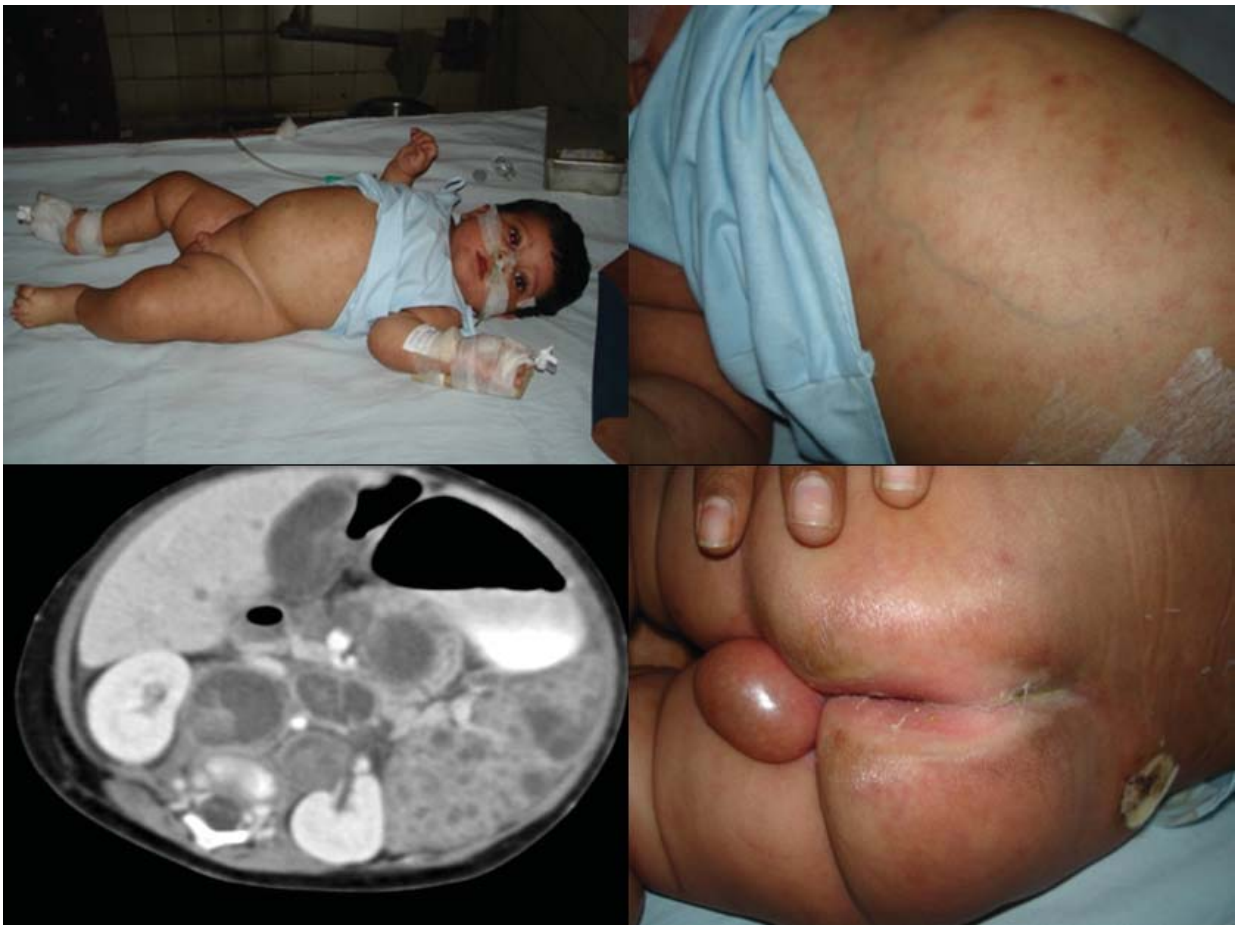
Frequency in SCID: >1/2 SCID infants are γc chain or Jak3 deficient (T-, B+ NK-)

>1/3 SCID infants are RAG deficient (T-, B- NK+)

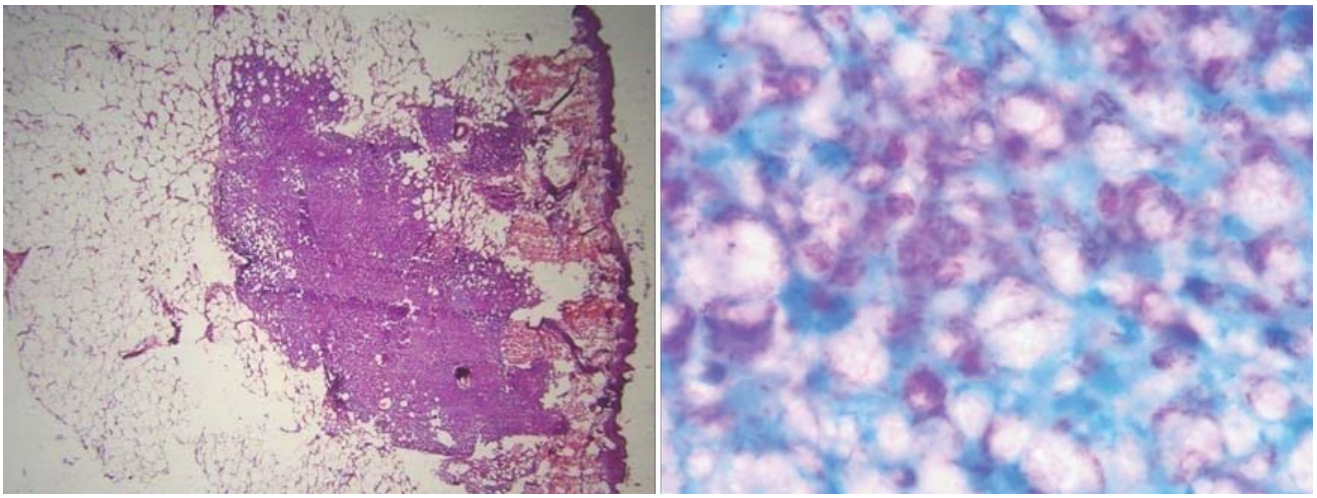
ADA def. are about 10-15% (T-, B- NK-)

In all the above 3 causes contribute to 98% of cases of SCID.

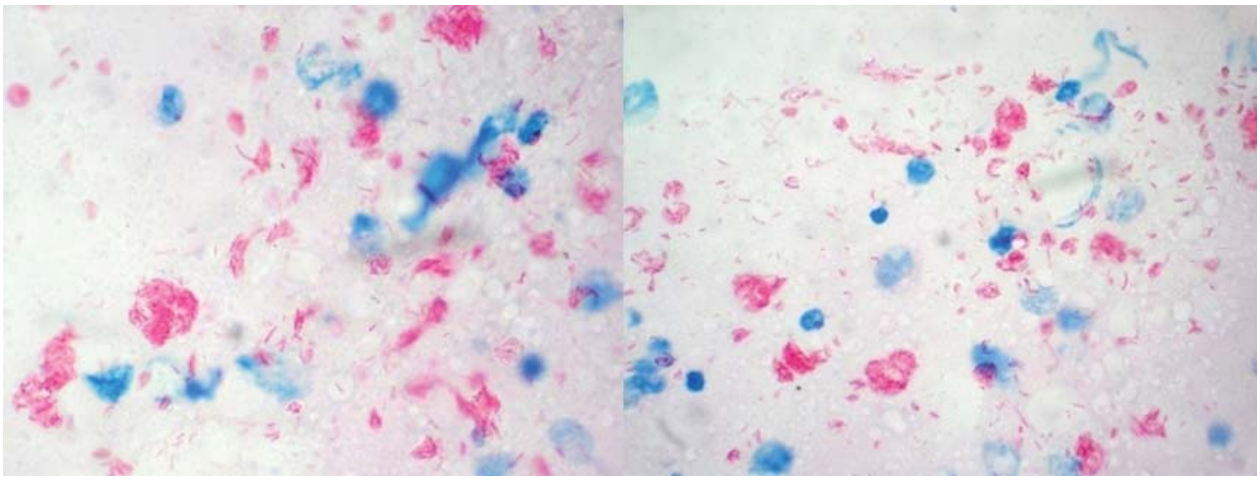
Pic 20, 21 & 22 show a case of SCID with disseminated BCGosis.



Pic. 20: Images show child with oedema, cutaneous granulomatous nodular rash, perianal fungal infection and imaging studies showing a massive hepatosplenomegaly with hypodense lesions in the spleen and large necrotic LN in the mesentery. The child was 5 months old & a disseminated BCGosis with SCID was suspected.



Pic. 21: Skin biopsy of the above child showing you a well formed granulomatous lesion and AFB staining of skin showing abundant amount of AFB (BCG).



Pic. 22: show AFB staining of Bone Marrow showing abundant amount of AFB (BCG).

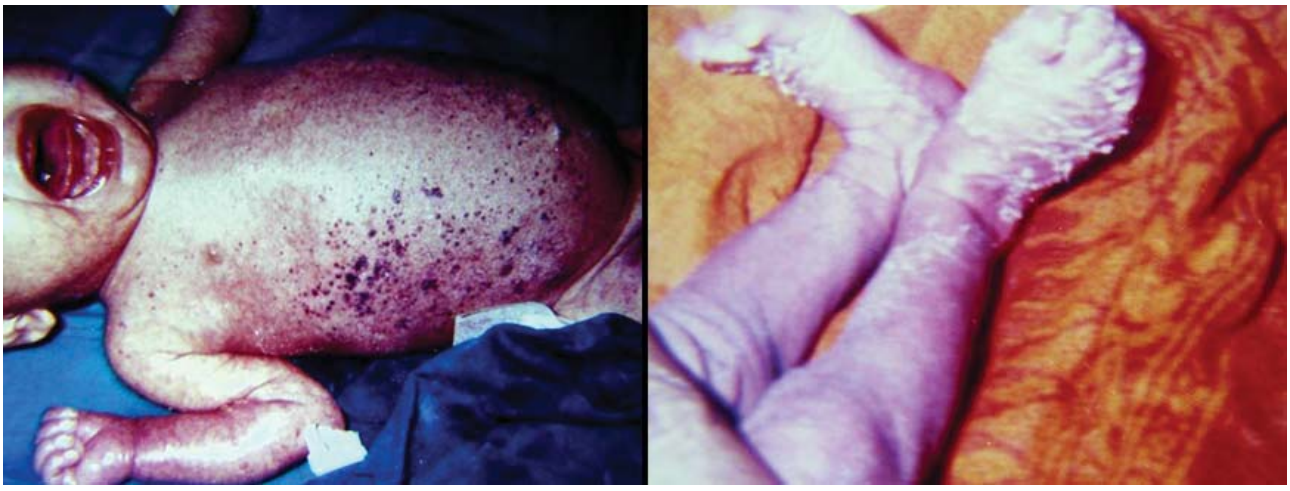
Consequences of SCID:

- Death if untreated (stem-cell transplantation, or replacement of the faulty gene), by 6-12 months of age before the onset of opportunistic infections. The most useful test for suspecting SCID is Absolute Lymphocyte Count (ALC) from birth and compare it with normal values for the population. In Mumbai we are doing this and the results are awaited in the next few months. Consanguinous marriages and previous male sibling death help in suspecting the diagnosis.

HLA (Human Leucocyte Antigen) Bare Lymphocyte Syndrome (BLS), Major Histocompatibility complex Class II (MHC Class II).

MQ, is a 7 month old male He presented with a complaint of 10 – 12 stools per day, for last 20 days. He had frequent episodes of diarrhoea almost since birth. He weighed 2.5 kgs with severe failure to thrive (FTT). At 6 mths of age he had pneumonia and at 7 months erythroderma with desquamation & hepatosplenomegaly. He later developed Rt. axillary lymphadenopathy. His skin biopsy showed plenty of histiocytes, lymphocytes, eosinophils which was diagnosed as psoriasis. 4 days later rash progressed to involve the entire abdomen and upper thigh and became purpuric. His investigations revealed a Hb of 8.0 gm/dl, WBC of 56,800/mm^3, poly 87% lymphocytes 9%, Monocytes 4% and platelets of 26 x 10^9/L. B M Aspiration showed a depleted red cell line suggestive of PRCA (Pure Red Cell Aplasia). LSSA revealed a T+, B+, NK+ SCID; HLA DR (HLA Class II) was absent.

HLA class II presents microbial Ags. To CD4 T cells so that there is an adaptive response. HLA class II defect presents at 4 months & death occurs between 6 months to 5 yrs, from bacterial, viral or fungal infection.



Pic. 23: Show extensive skin peeling which clinically appeared to be Omenn syndrome. Skin biopsy however was reported as psoriasis. BLS (Bare Lymphocyte Syndrome) was diagnosed on work up. Image courtesy Dr M R Lokeshwar.

HLA class II deficiency is an autosomal recessive disorder. The genetic mutation causes a defective transcription of MHC II genes. Manifestations in 30 children with MHC class II deficiency (BLS) were protracted persistent diarrhea in 86% of babies early in infancy, oral candidiasis 30%, pneumonias in 86%, 63% viral infections and FTT in 73%. One child had psoriasis; no Transfusion associated GvHD occurs. Autoimmune cytopenia is seen in 6%. The lymphocyte subsets, shows a T+, B+, NK+ phenotype. In 34/39 babies CD 4+ T cells are decreased along with B cells. Serum immunoglobulins are low with IgG decreased in 24/30, IgM decreased in 28/40 & IgA decreased in 31/40 cases.

The patient expired with pure red cell aplasia (due to Parvovirus B19)

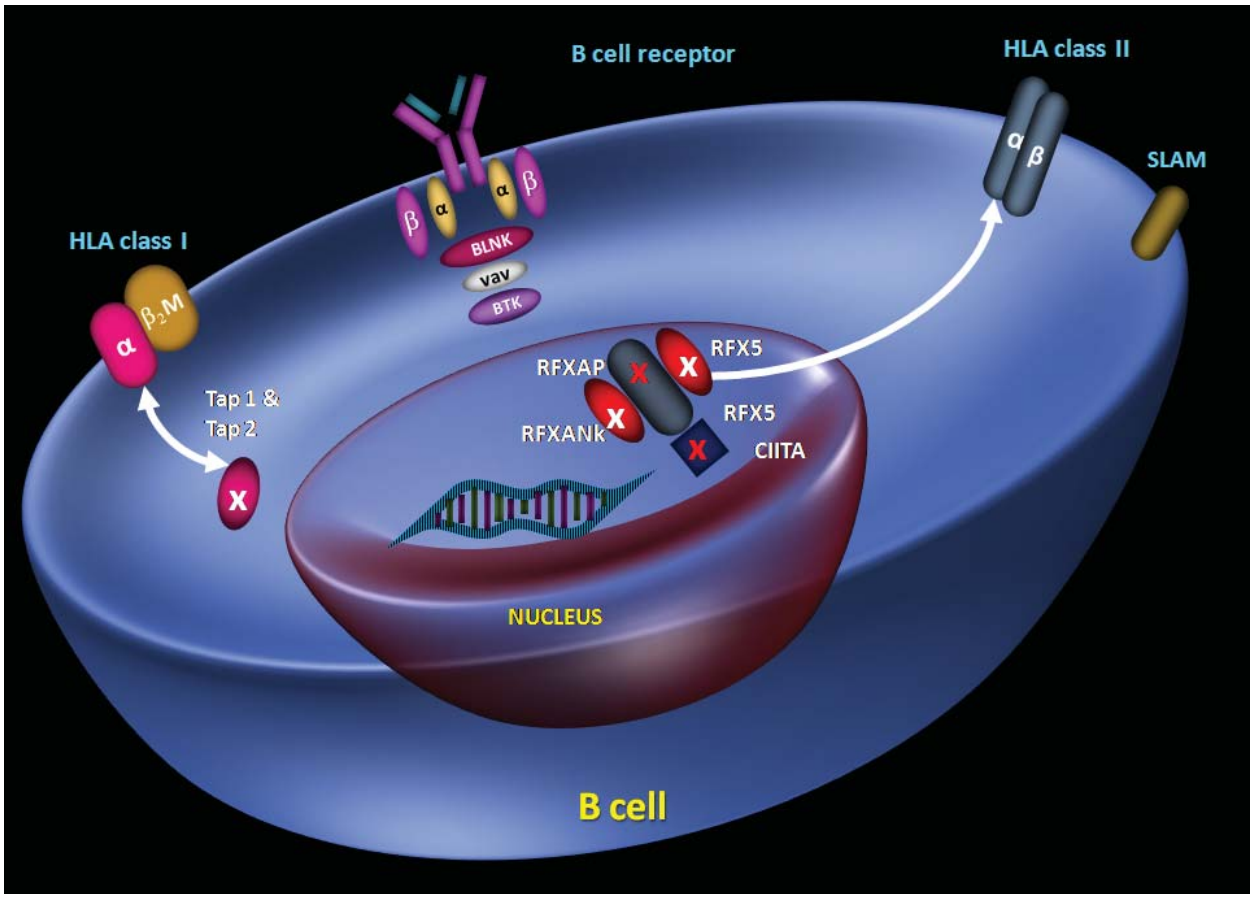
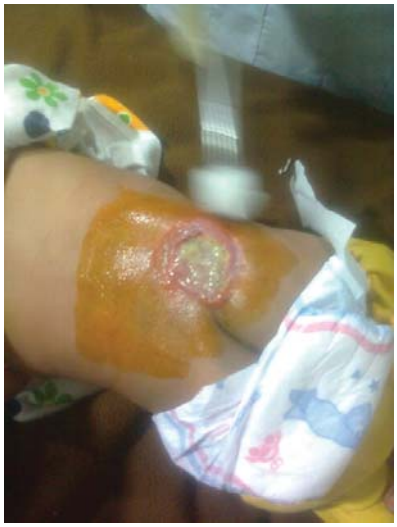


Fig. 15: Shows that defect in HLA Class II molecule is due to defective transcription factors (TF) which control the expression of HLA class II gene, viz RFXAP, RFX5, RFXANK, CIITA. Defects in all these transcription factors are described and result in Bare Lymphocyte Syndrome. HLA Class I to deficiency is due to defect in peptide loading proteins Tap 1 & 2.

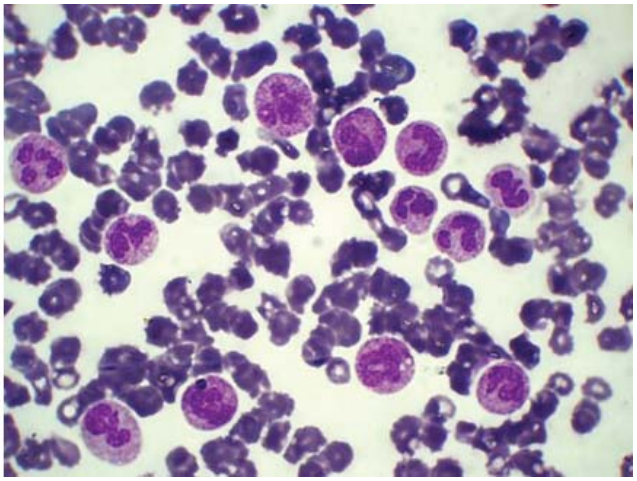
Phagocytic defects

Leucocyte Adhesion Defect

LAD can be I, II or III (commonest is LAD I).



Pic. 24: of a child with a large bone deep ulceration at BM aspiration site; child had persistent neutrophilic leucocytosis and otitis media. Immune work up showed a complete deficiency of CD 18 confirming LAD (Leucocyte Adhesion Deficiency) Type I.



Pic. 25: of a 15 day old child with extensive subcutaneous infection & Neutrophilic leucocytosis secondary to LAD type I

P.S was admitted on 6/12/1995 at the age of 3 months with intermittent fever for 1 month, ulcers over chin, sacral area with cellulitis, ear discharge first from R. ear and then L. ear

There was first degree consanguinity in family and the elder sib was alright at 3 yrs.

There were multiple infections, starting at birth with umbilical infection, ear infection, pneumonia and pyrexia of unknown origin.

There was history of delayed separation of the umbilical cord

Physical Examination: There was FTT with wt gain of 1 kg since birth; child was febrile (100^0F) with skin ulceration on chin and sacral area. The R.R was 36/min liver 5 cms & spleen 4 cms. Cardiac rate was 110/min with occasional crepitations on auscultation.

Laboratory & clinical course is shown in table (6).

Laboratory data:

Table 6: Show clinical & Laboratory data of patient with LAD type I

Age	Date	Hb	Wbc	P	L	M	Eo	Plts	Sign and symptoms
1 mth	1994 31.12		61.0	17	81	1	1	Adeq	Chest XR RUZ pneumonia
2 mth	1995 7.1		71.0	73	26	1	0	Adeq	Multiple antibiotic from 6.1.95 to 8.3.95
	18.1		63.0						
3 mth	1995 7.2	5.1	39.9	68	22	8	2	Adeq	Fever 101.5^0 F SGPT 46 IU, Uric Acid 7.3 mg/dl, chol. 248 mg/dl, Alk phos. 33 IU, albumin 3 gm/dl, Cr 0.5%
	13.2	6.1	74.4	79	18	5	2	Adeq	Fever 103^0F for 1 day
	20.2	4.6	67.7	83	10	5	2	Low	Transfused 5 times
	23.2	7.5	81.4	78	13	6	3	65	BM: normal
4mth	6.3	4.6	47.7	76	18	5		Low	Chest X-rays: RUZ pneumonia $P0_2$ 41%, PCO_2 36, O_2 Sat.79%, HCO3 24.
	8.3 1995	Expired							

Serum IgG & IgM were increased and Ear swab culture grew Staphylococcus aureus & E coli. CSF: 1 cell, proteins 12 mg/dl and culture sterile. There was no growth on blood culture. HIV and VDRL was negative, HBsAg was weakly +ve. Immune work up (NBT, C3 & C4) was normal.

In **LAD I** gene that encodes integrin (CD18) in neutrophils for firm adhesion is mutated. Thus neutrophils can roll but not firmly adhere to blood vessel endothelial cells and cannot exit circulation and go to the site of infection Fig.16 & 17. The patient had a typical history and marked polymorphonuclear leucocytosis with or without infection (Pic 24 & 25).

There is an absence of pus formation at the sites of infection. Frequently infections are due to S. aureus and gram –ve enteric organisms, or fungal, and if they survive infancy they have severe gingivitis and periodontitis.

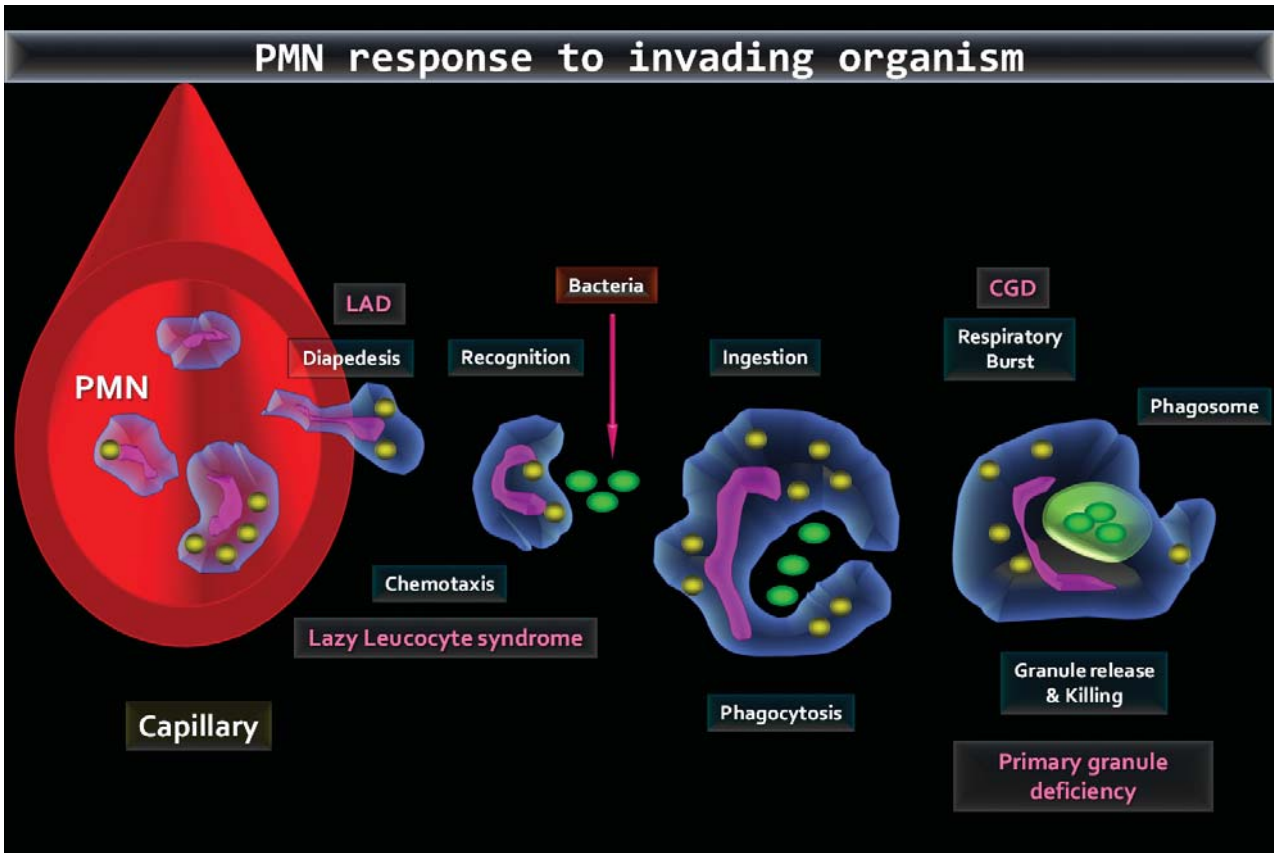


Fig. 16: show the innate response of PMN (Polymorph) to an invading organism. The PMN has to leave the circulation and migrate to site of infection, recognize the organism and phagocytose it. Release of antimicrobial PMN granules into the phagosome then activate the NADPH Oxidase respiratory burst to kill these organisms. Also shown are diseases resulting from defects at different steps in this process

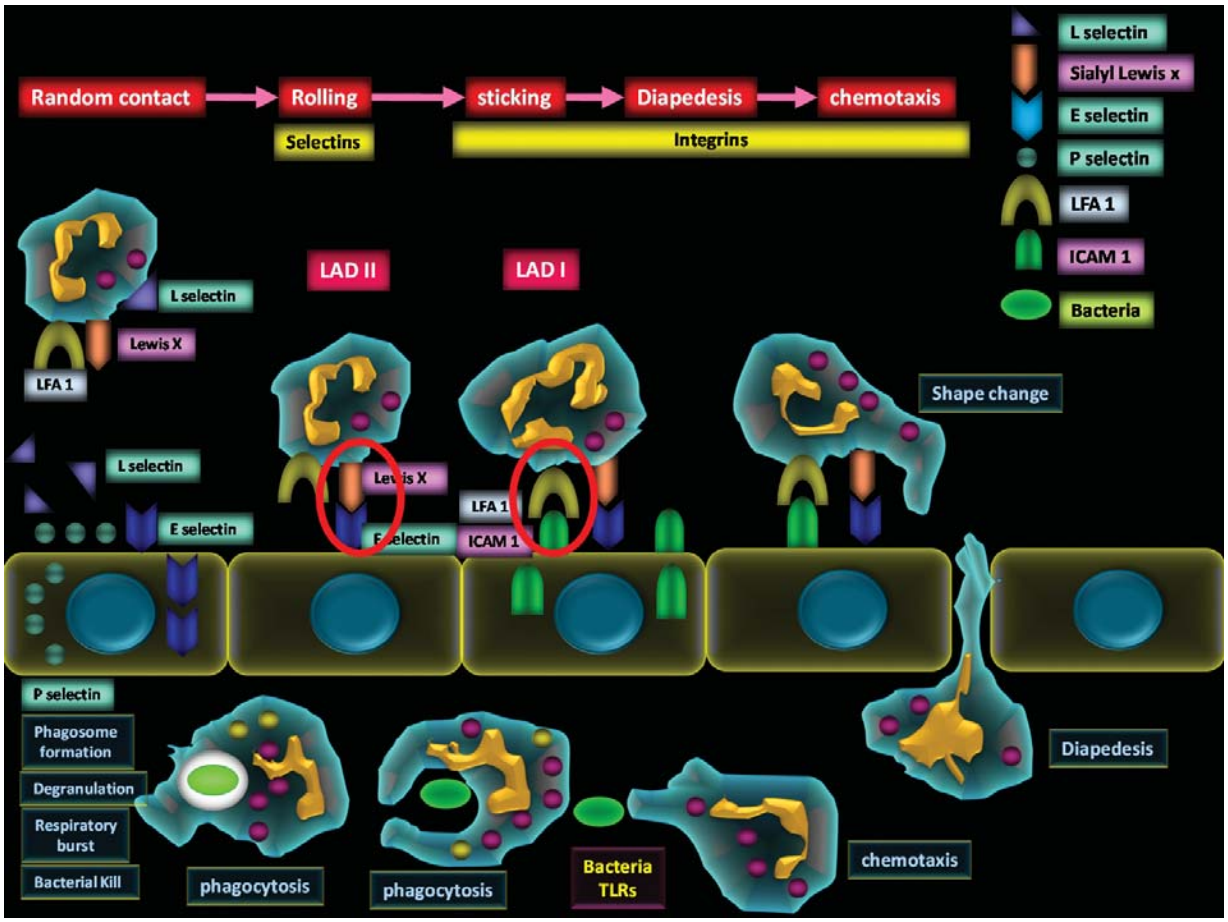


Fig. 17: In response to bacterial invasion the polymorphs need to migrate to the site of infection. This process in guided by chemokines and adhesion molecules. Adhesion molecules are expressed by the inflammed endothelium in response to inflammatory cytokines IL1 & TNFα that are secreted by innate PMN cells on recognition of bacteria. The process is orderly and orchestrated by sequential expression of adhesion molecules. The first process is rolling for which P selectin Lewis X & L selectin are required. Rolling arrests a fast flowing neutrophil & tethers it to the endothelium. Defect in Lewis X results in LAD type II. The next step is sticking and the integrins ICAM 1 (Intercellular Adhesion Molecule) and LFA 1 are important. Defect in adhesion molecule LFA 1 results in LAD type I. LFA 1 is a hetero dimer and made up of α & β chain. The β chain is also shared by other adhesion molecules. LAD I is a defect in β chain which can be diagnosed by flow cytometry using anti CD 18 antibodies. After sticking the neutrophil undergoes diapedesis and egresses out of the circulation. Subsequently it will migrate to site of infection with help from chemokines. (attractants)

PMN defects can be (1) Quantitative or (2) functional.

(1) Neutropenias or (2) Functional PMN defects as in (a) CGD or (b) Specific Granule Defects

(1) Specific genetic disorders in the Polymorphonuclear cells (PMNs) like Neutropenias are rare, hence acquired causes (e.g. drug or infection induced) & autoimmune neutropenia should be ruled out.

The hereditary disorders are: In 50-70% ELA2 gene defects: Severe Congenital Neutropenia (SCN) & Cyclic SCN; & HAX1 gene defect in 30%, Kostmann Syndrome; the Albinos CHS (Chediak Higashi Syndrome), HPS (Hermansky Pudlak Syndrome) type 2; and others which are rare. Most SCNs (90%) respond to G. CSF.

For diagnosis: persistent (or cyclic) Absolute Neutrophil Count (ANC) of < 500 / mm^3 in blood.

If there is no response to G. CSF, be alert to the develoment of leukemia (MDS/AML)

(2) (a) Recurrent severe infections (Pneumonitis, abscesses) due to bacteria & fungi fungi in functional PMN defects e.g. CGD.

(2) (b) Specific granule deficiency or defects in PMNs.

This is very rare. As the developing PMNs differentiate in the BM from the Myelocytes to mature PMNs, they acquire various specific granules. Due to a congenital absence of these specific granules the PMNs are unable to respond to & destroy bacteria.

Hyper IgE syndrome (HIES) and Hyper Eosinophilia syndrome (HES)

HES:

Eosinophilia can occur in many allergic and parasitic diseases, or it can occur in diseases of the immune system e.g. HIES / Job syndrome, WAS (Wiskott Aldrich Syndrome), Hyper IgM & IgA deficiency. Living in Mumbai, an ex-fishing village, where eosinophilia & parasitic infections. are commonly seen, there is always a dilemma as to whether one is dealing with a HES or HIES. In both, increased Absolute eosinophil count (AEC) and an elevated IgE levels. An AEC of 350-650 / mm^3 (or 1-3%) is normal; <1500/mm^3 is mild, 1500-5000 is moderate and >5000 / mm^3 is severe eosinophilia. In HES in peripheral blood, > 1500 / cumm for > 6 months and no other cause of a high AEC is considered as diagnostic. There is a persistent blood and tissue eosinophilia, later leading to organ damage. In 2009 HES was divided into Myeloid HES and Lymphoid HES. In the latter, L-HES, the cause of HES is a cytokine 1L.5, produced by abnormal T-cell clones and frequently resulting in elevated IgE, skin involvement, a history of atopy, and a good response with corticosteroids. In M-HES, a form of chronic eosinophilic leukemia, a fusion of FIP1L1 and PDGFRα genes is found, often in males with cardiac involvement, & very high eosinophil counts (>100,000 / mm^3), hepatosplenomegaly, increased vitamin B12, & decreased Hb or platelets.

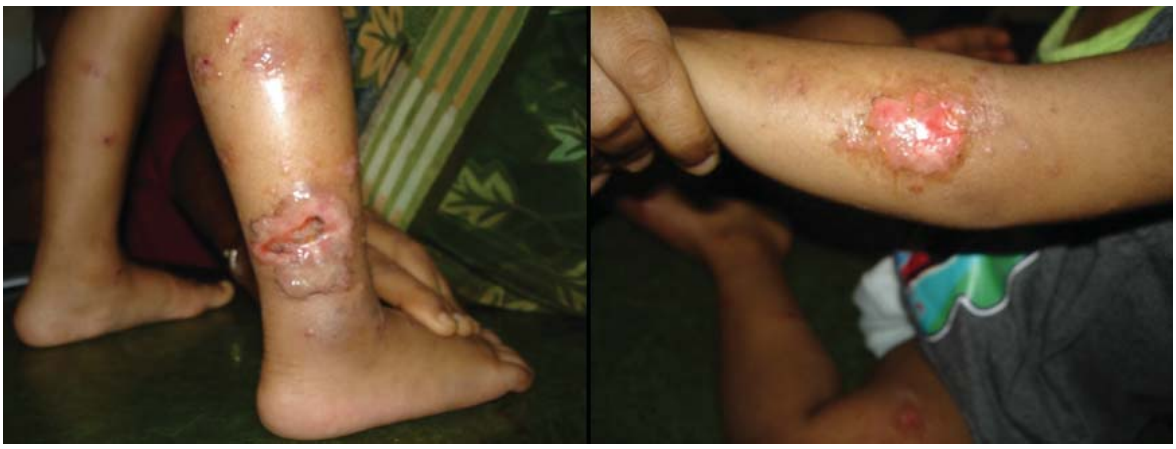
However, serum IgE concentrations can be very high in atopic eczema, asthma and other conditions eg.25, 600 ng/ml in atopic patients. We have seen 14,300 ng/ml in ascaris infections. Serum IgE is

very low in utero and climbs after birth .This creates a problem in diagnosis during infancy hence if the serum IgE is above 10 times the age adjusted normal, that value may be taken as diagnostic for HIES. However IgE levels fluctuate and may touch normal without a change in the clinical condition of the patient. Eosinophilia is seen in 90% of the patients at least 2 SD above normal, usually with AEC >700 eos / mm^3.

HIES:

Signs and Symptoms: Dermatitis, boils, pneumatocele forming pneumonias, high serum IgE levels (10 x N for age), retained primary dentition and scoliosis, recurrent fractures because of osteopenia, hyper extensible joints and coarse facies.

Organisms: Recurrent Staph abscesses of the skin typically described as cold abscesses; Pic. 26, & in lungs, joints, other organs, Lung cysts are often super infected with Aspergillus, Candida, Haemophilus influenzae and gram –ve bacteria.



Pic. 26: child with HIES with classic cold abscesses involving the skin. HIES is due to mutation in STAT3 protein & the infecting organism is most likely Staphylococcus aureus

Inheritance is both autosomal dominant & sporadic with mutations in STAT 3, that causes HIES. Mutations in Tyk2 also causes HIES (see Fig 18).

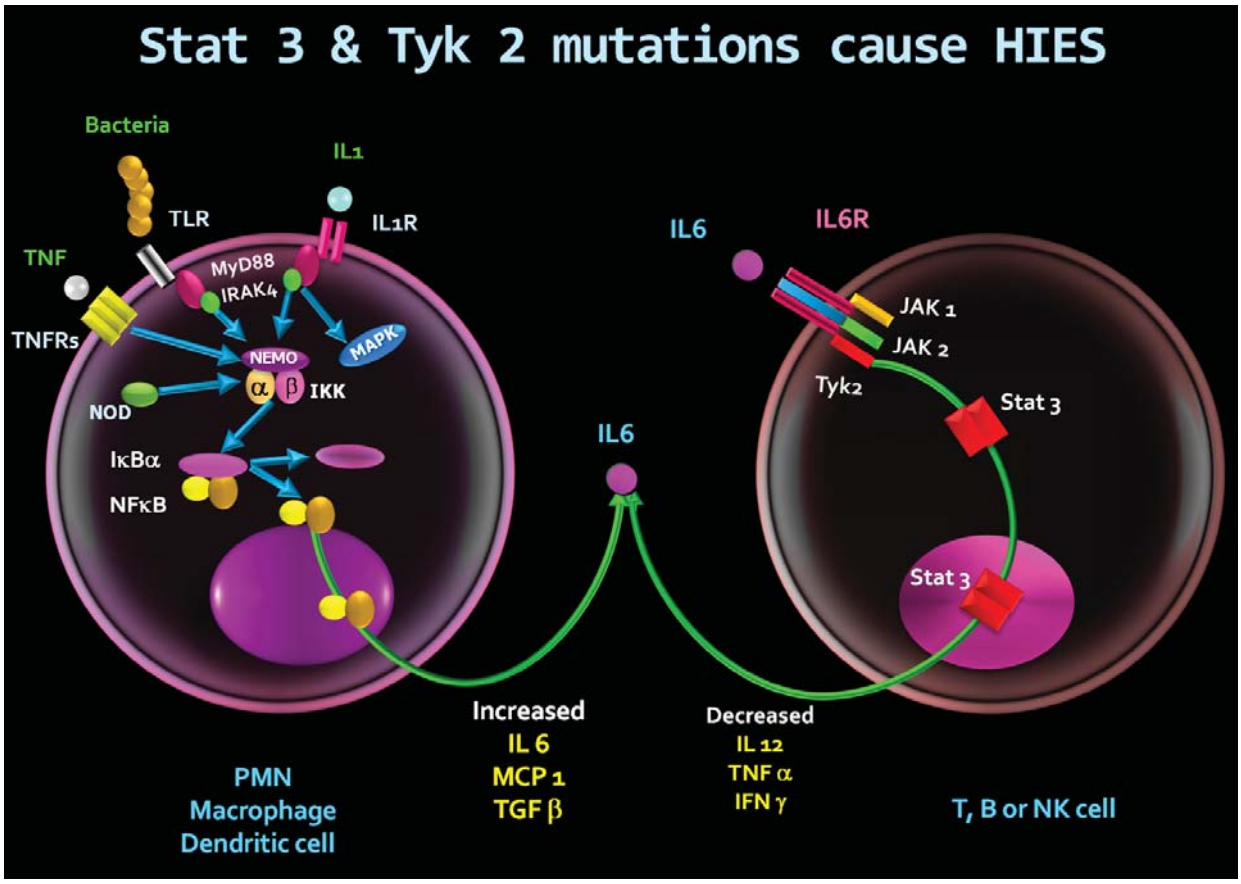


Fig. 18: Dominant negative mutations in STAT3 in lymphocytes result in (Autosomal Dominant) AD-HIES. It could be sporadic or familial. Many cytokines transmit signal through the JAK STAT pathway. STAT3 is a transcription factor that is involved in transducing signal from many cytokines like IL6, IL10, IL21, IL22, and IL23 and plays a role in angiogenesis, cancer, wound healing & immunity. It dimerizes after activation by JAK and result in increased secretion of IL6 & MCP1 (proinflammatory cytokines) and TGFβ which is an anti-inflammatory cytokine. Production of IL12, TNFα & IFNγ is suppressed. Clinically in patients of HIES there is both excessive inflammation (e.g. lung pneumatocele) & inadequate inflammation (e.g. cold abscesses of skin & susceptibility to infection). The helper CD4 + cells Th17 is completely absent as STAT3 is integral to Th17 differentiation of CD4 cells. Th17 recruit neutrophils and also up regulate antimicrobial peptides for host defence. Th17 cells are also important for IL22 which is critical for beta-defensins secretion which is important for control of Staphylococcus aureus infection. This explains special susceptibility of HIES to Staphylococcus aureus. Since C reactive protein is produced in response to IL6, it is often reduced during acute infection in HIES. Extreme elevation of IgE in HIES however remains poorly explained

Hyper IgM syndrome (HIGM)

TF is a 3 yr 10 month old female child, the first born of a 3rd degree consanguineous parents. She was admitted on 24.1.06 for fever cough and dyspnoea for 10 days. She has had similar complaints since 7 mo of age for which she was hospitalized 3 times.

(Hyper IGM Syndrome) 3 yr 10 months old female.

Physical exam: she was febrile with bilateral pitting oedema, bilateral crepitations on lung auscultation, and a maculopapular rash over the abdomen; Liver 6 cms and spleen of 20 cms.

LAB evaluation was as follows; Hb 9.6 g/dl, WBC 4.84 x 10^9/L; Polys 59.7% , Lymphs 36.6%, Mono 2.2%, Eos 2%, Baso 2%, plts 80 x 10^9/L; retic 7% (Coombs negative). Increased retic and low platelets resolved within 1 week. IgE was 2.01 IU & HIV was negative

Chest X ray showed paratracheal lymphadenopathy; & lung parenchymal changes were suggestive of interstitial edema. Lung biopsy on 11.2.06: showed interstitial lung disease consistent with desquamating interstitial pneumonia.

B.M was hypercellular with trilineage hyperplasia; ANA was negative. Ultra fast CT scan of thorax and abdomen showed bilateral pleural effusion (R > L); bilateral basal consolidation, an enlarged para tracheal node pericardial effusion, and gross hepatosplenomegaly (spleen > liver). Pleural fluid examination was normal.

Second admission was at BJWHC on 13.06.2006 at 4 yrs of age. Her wt was 14 kg & height 96 cms, Lt axillary LN was palpable 1 x 1 cm significant. Liver was 4 cms and spleen was 10 cms enlarged.

CBC was normal. Igs: IgG 0, IgA 0 & IgM 900 mg/dl.

LSSA (Lymphocyte Subset Analysis) on 14.6.2006 had an ALC of 1740, low B cells, increased CD 4+ T cells & low CD 8+ cells. HIV was negative.

There are 4 types of HIGMs. However all 4 may be recognized clinically but molecular analysis is needed for genetic counseling, carrier detection and a definitive diagnosis. (Fig.19)

1) X-linked HIGM (HIGM1) Defect in CD40L (CD154) on T cells.

   Male, IgM is normal or high but IgG and IgA are very low; onset is during first 2 yrs of life; clinical presentation is with recurrent pyogenic infections in 87.5%, including otitis media, sinusitis & pneumonia (82.1%), tonsillitis, diarrhea (55.3%), and oral ulcers (44.6%).

   Liver / biliary tree with sclerosing cholangitis (19.6%), hepatitis (16.17%), cirrhosis (10.7%), sepsis (14.3%), & arthritis (10.7%).

   CNS meningitis / encephalitis (8.4%), encephalopathy (3.6%), tumour (3.6%), Neutropenia (67.8%) which is chronic (44.6%), cyclic (12.5%) or episodic (10.7%), and anemia (32.1%).

   Most prominent clinical feature is the increased incidence of opportunistic infections with cryptosporidium, Pneumocystis jiroveci, Mycobacteria and CMV. This is because the basic defect is in T-cells although hyper IgM sounds like a humoral defect.

2) NEMO (HIGM4) a genetic syndrome with anhidrotic ectodermal dysplasia associated with an immune defect in boys. Females show incontinentia pigmentosa, lethal for male fetuses. B & T cells and DCs (Dendritic Cells) are affected.

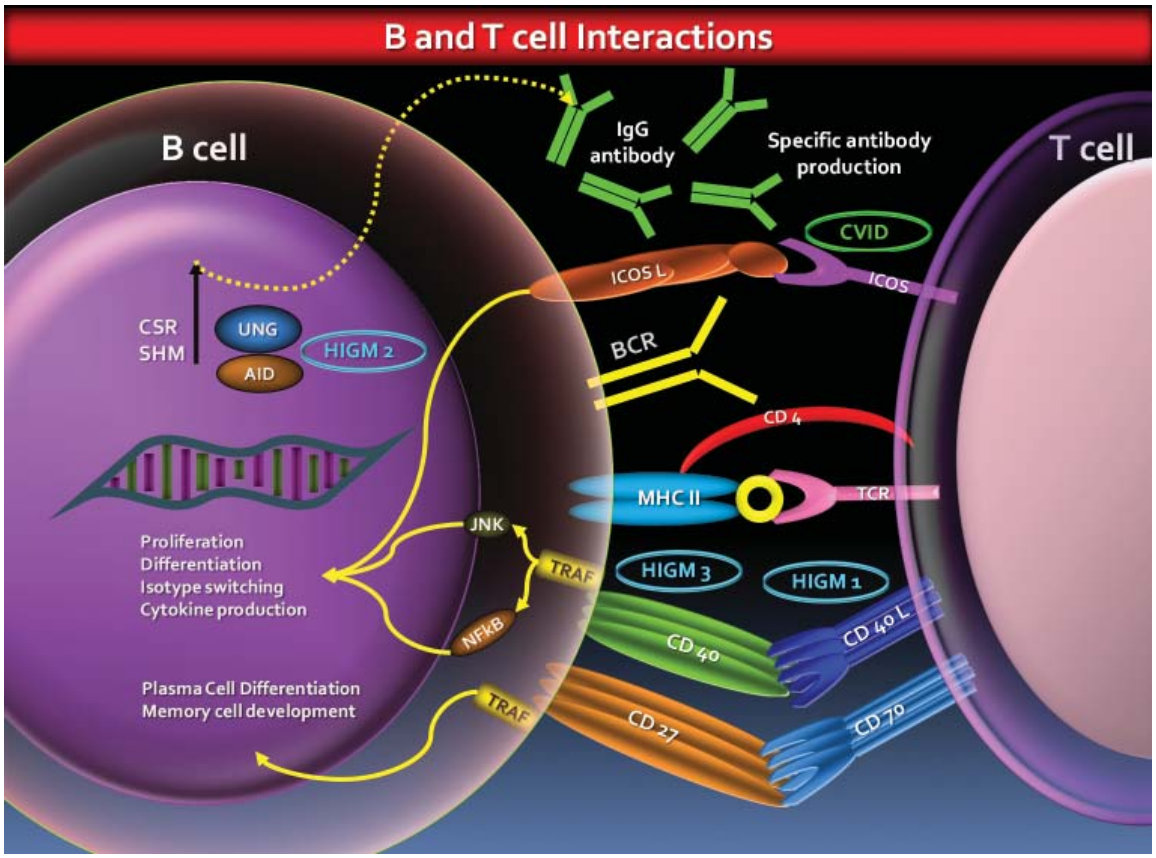


Fig. 19: T cells provide crucial help to B cells in the lymph node germinal centre (GC) for class switching from IgM to IgG, IgA or IgE antibodies. Once the B cell recognizes an antigen with it BCR (B Cell Receptor) which has an IgM or IgD surface molecule, it enters GC reaction. In the GC is an orderly sequence of events takes place. The purpose of Germinal Centre reaction is to find the best fitting antibody for the antigen. There is initially an intense proliferation of B cells during which there is Somatic Hypermutation (SHM) and Class Switch Recombination (CSR) to provide effective immunity. Other molecules are also important e.g. CD 40 ligands (CD 154) on T cells & CD 40 R on B cells, ICOS L & ICOS, CD27L & CD27. Also shown are defects resulting in HIGM & CVID.

3) (HIGM2) Activation Induced Cytidine Deaminase (AID) defect resulting in lymphoid hyperplasia (LN+) although there is a defective Germinal Centre formation and defective CSR (Class Switch Recombination). Inheritance is autosomal recessive therefore both males and females affected.

4) Mutations in CD 40 (HIGM 3): CD 40 is absent on the B cell surface. The B cells are thus intrinsically abnormal and cannot interact with normal T-cells which have CD 40 ligand to act on CD 40 on B cells.

Final Diagnosis of our patient: HIGM 2 / or HIGM 3 i.e AID (Activation Induced Cytidine Deaminase) / UNG ((Uracil DNA glycosylase).

Conclusion: our patient fits in with AID or UNG defect. In both these conditions serum IgM is increased & not normal; in AID defect it is increased in 95% while in UNG defect it is increased in all (100%).

In AID defect serum IgA & IgG are undetectable in 83% & 85% of cases respectively while, in UNG deficiency it is undetectable in 33% & 33% of cases respectively.

HIGMs are due to defect in either T-cells or B-cells: 2 subtypes of T cell defects & 2 B cell defects. (Fig. 19)

Although B cells are numerically normal, the interaction between B and T cells is faulty, leading to impaired switch over from IgM to IgG, IgA and IgE.

Two enzymes AID and UNG are needed for the switching in L.N germinal centre (fig. 19).

Genetically HIGM is heterogeneous shows mutation in 2 genes on the X-chromosome CD 40L (CD 154) expressed on T cells as shown in Fig. 19 and NEMO; and 2 genes expressed an autosomal chromosomes (AID expressed on B cells on Chromosome 12, & CD 40 on chromosome 20 expressed on B cells.

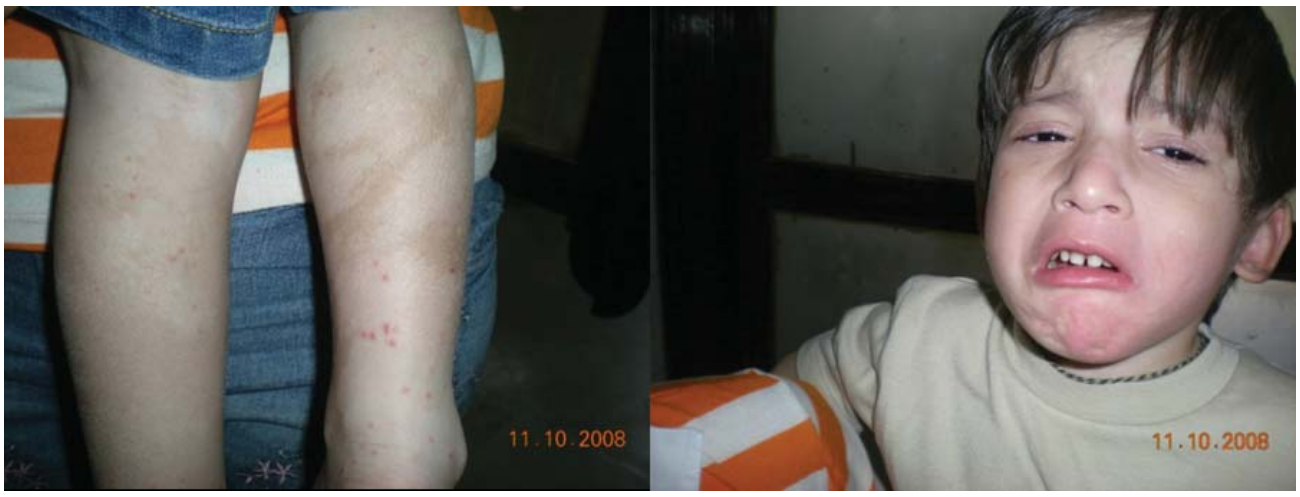
PIDs associated with Albinism

I. Chediak-Higashi Syndrome (CHS):

C.R.Y. a 3 mo old baby complained of fever for 15 days

Physical examination showed Heterochromia iridis, umbilical hernia and the liver 3 cms was palpable.

CHS is an autosomal recessive disorder due to defect in CHS 1/LYST gene, seen in all haematopoietic cells.



Pic. 27 : shows partial albinism & hair changes typically seen in Chediak Higashi Syndrome.

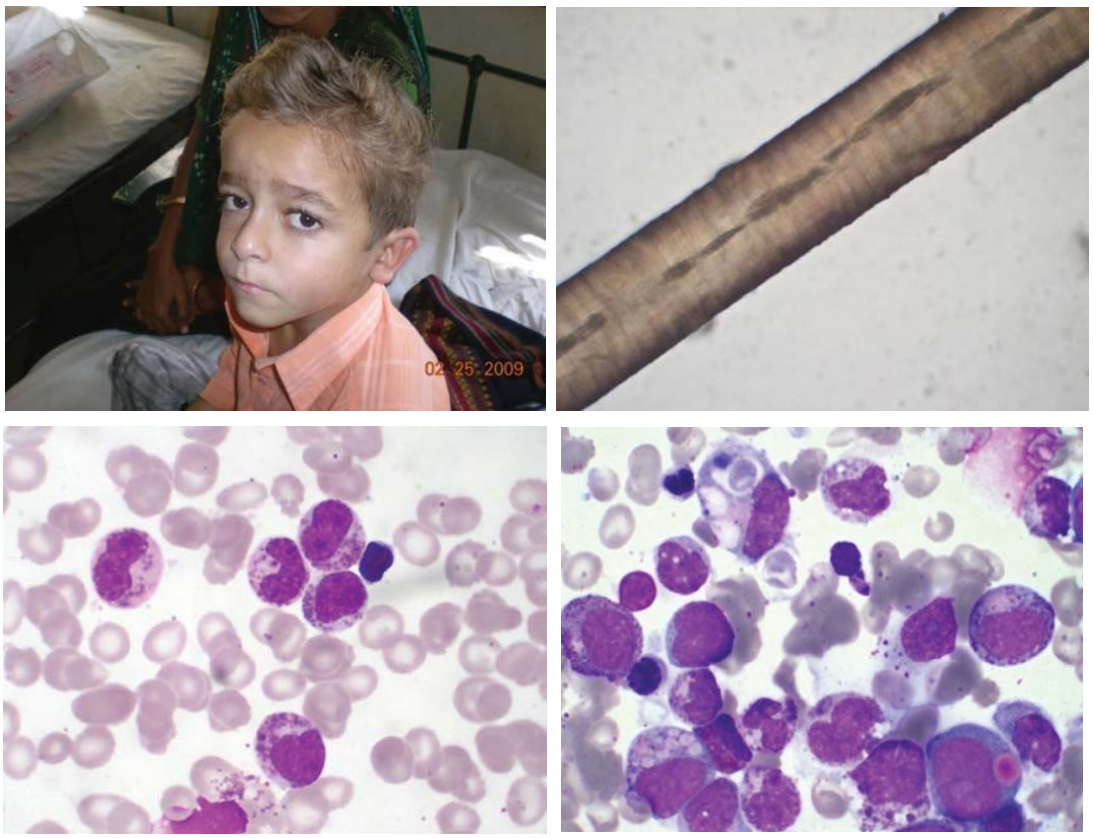
CHS shows signs of immune deficiency, frequent pyogenic bacterial infections, a bleeding tendency, variable albinism, initially partial with hair grey to white, & frequently, altered eye pigmentation.

Progressive neurological dysfunction including weakness, ataxia, sensory defects and progressive neurodegeneration occur as child grows during the second decade of life.

A Lymphoproliferative disorder implies an accelerated phase in CHS. Lymphoproliferation is from lymphocyte infiltration in major organs of the body.

Diagnostic sign: In CHS large granules are seen in hematopoietic cells which do not function like normal granules in blood cells, & in hair melanosomes (Pic. 28).

Platelet dense granules defect result in a mild coagulation defect resulting in bleeding; defects in cytolytic secretory granules of phagocytic cells control bacterial infections & cytotoxic T lymphocytes in killing virally infected cells, resulting in infections of respiratory tract and skin. If the subject does not succumb to infection, CHS children go into an “accelerated phase” due to uncontrolled T cell and Macrophage activation in response to a viral infection, or a T cell function defect resulting clinically in Hematophagic Lymphohistiocytosis (HLH) with multiorgan failure and death (Pic.27 & 3 pg. 14).



Pic. 28: Show cell with large inclusion granules and a macrophage showing erythrophagocytosis and empty phagosomes. HLH is commonly seen in accelerated phase of CHS. Also see fig.6 for role of LYST gene in granule maturation during process of granule exocytosis. Also note albinism & characteristic hair changes in another case of CHS. (Pic. above)

II. Griscelli Syndrome:

Pic. 29: Note the typical hair changes seen in Griscelli syndrome classically described as ashen grey and sparse & abnormal melanin distribution in hair. Pic. courtesy Dr M R Lokeshwar,

There is a variable cellular immune deficiency, with a decrease in T cells and NK cell cytotoxicity

There are 3 types of Griscelli syndromes & the genes mutated are Myosin Va, RAB27α and MLPH in GS 1, GS 2 & GS 3 respectively. Gene for Myosins Va is located on chromosome 15q21. These proteins are shown in Fig 20. They are concerned with membrane transport and organelle trafficking inside a cell.

Patients typically have ashen grey hair due to sparse and abnormal melanin distribution in hair see pic 29. Patients are prone to recurrent acute HLH, in which the trigger is probably a virus infection. Fever, hepatosplenomegaly, CNS infiltration by activated lymphocytes and macrophages, coagulopathy and pancytopenia, the hall marks of HLH. Death generally occurs within 5 yrs, from recurrent infections or CNS disease.

RAB27α is also expressed in NK cells & Cytotoxic T Lymphocytes and is important in granule exocytosis. Thus GS2 is the only type of Griscelli syndrome associated with HLH see Fig 6.

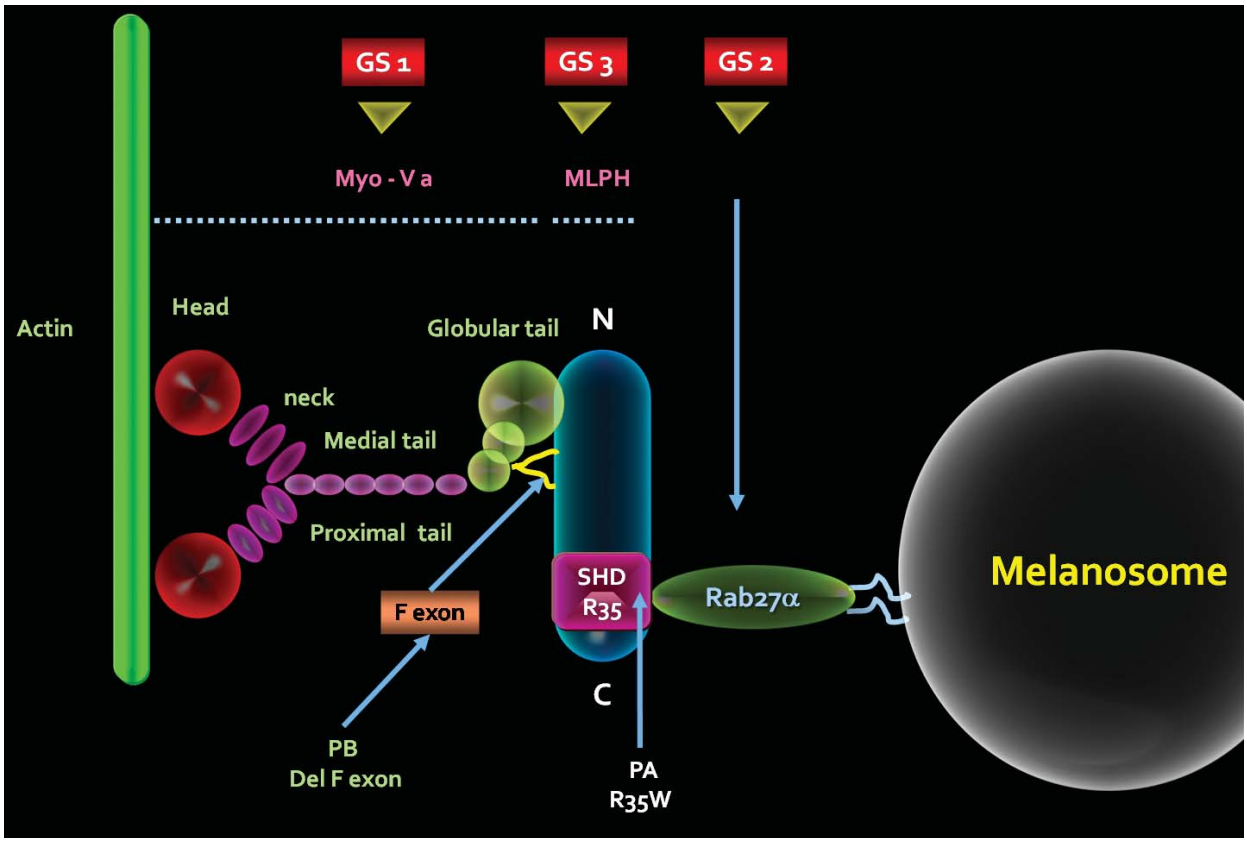


Fig. 20: Three proteins play an important role in melanosomes & granule trafficking within the cell. Myosin-Va is a large protein which has a head, neck, a medial tail, proximal tail and a globular tail. With its globular tail it binds to another protein MLPH which in turn binds to RAB-27α responsible for binding & docking of cytoplasmic granules like melanosomes. Mutations in Myo-Va result in clinical phenotype of Griscelli I which also has CNS manifestations. Mutations in MLPH result in Griscelli type III; while mutation in RAB 27α result in Griscelli type II. RAB 27α also play an important role in docking of cytotoxic T cell / NK cell granules which contain perforin and granzyme B. Hence only Griscelli type II with defect in RAB 27α result in HLH. Also (see fig 6, Pg. 12) for role of RAB27α in granule exocytosis.

III. Hermansky-Pudlak syndrome (HPS)

HPS presents with partial albinism due to faulty melanosomes resulting in occulocutaneous albinism.

Increase in bleeding time is due to platelet dysfunction. Dense granules in platelets are lysosome related organelles, necessary for normal blood hemostasis & important in vesicle formation + vesicle trafficking i.e for docking of vesicle to the membrane of cell as shown in fig. 6, Pg. 12.

HPS is an autosomal recessive disorder due to defects in seven genes. This dysfunction affects lysosomes and the 2 lysosomes related organelles are melanosomes and platelet dense body granules .

Auto immune lymphoproliferative syndrome (ALPS):

In every cell there is programmed cell death (apoptosis) (Fig. 21). ALPS is a defect in programmed cell death of lymphocytes resulting in accumulation of non-malignant lymphocytes in the lymph nodes or spleen early in life; a propensity to develop autoimmunity in 2/3rd of cases; & malignancies including Hodgkin's lymphomas.

The manifestations of ALPS are seen in following frequencies;

- Lymphoproliferation 100%, commonly presenting with a "bull neck"
- Lymphadenopathy 92%
- Splenomegaly 88%
- Hepatomegaly 72%
- Antibodies increased in 70%
- Coombs +ve 51%
- Thrombocytopenia 47%
- Neutropenia 23%
- Optic neuritis, Uveitis, Episcleritis 2-3%

Diagnosis is easily established by measuring T cell receptor (TCR) $\alpha\beta$ CD4-, CD8-, double negative T cells (DNT) in the peripheral blood by flow cytometry (LSSA: Lymphocyte Subset Analysis). Typically in ALPS, DNT cells are increased in range of 5-20%. In a normal child DNT cells are < 1%.

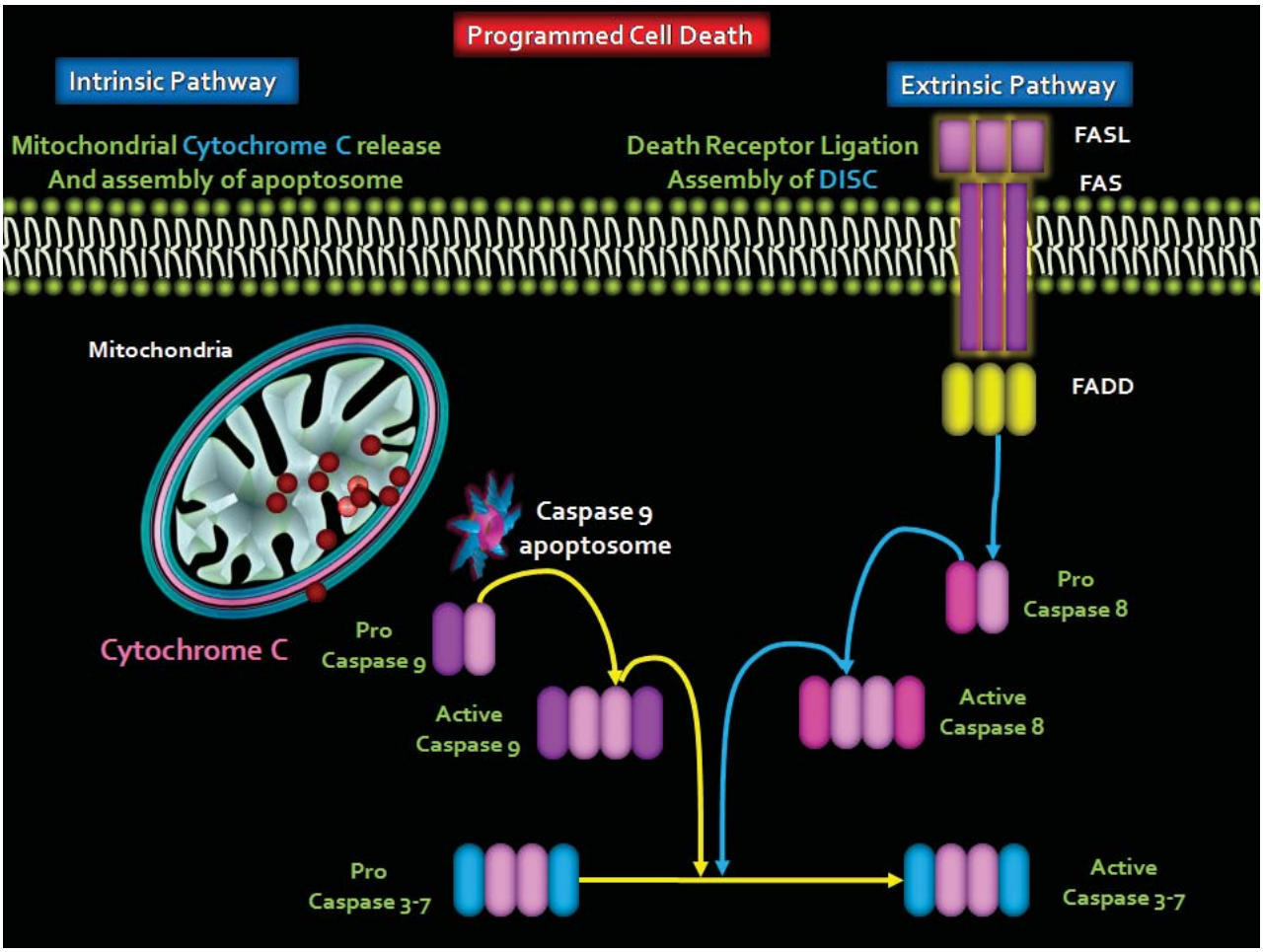
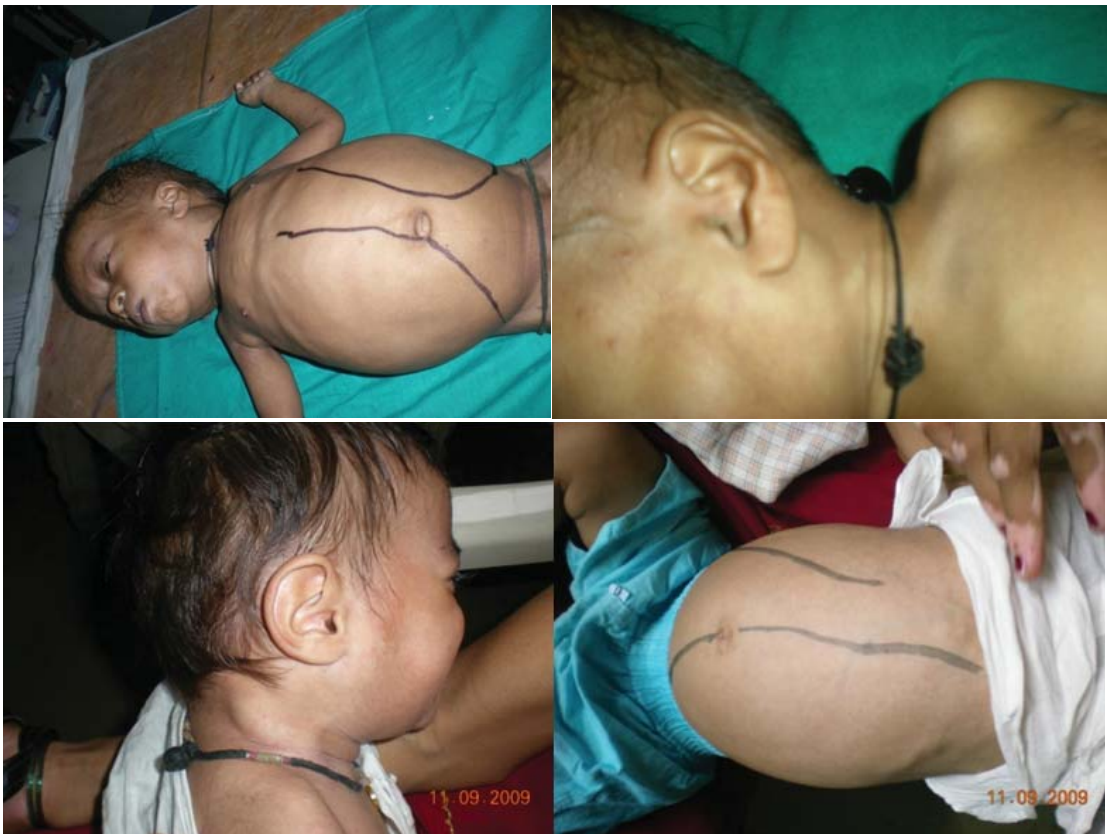


Fig. 21: All cells are programmed to die (apoptosis). Apoptosis results in activation of 3 pathways: (1) Perforin granzyme pathway is important in killing virally infected cells or tumor cells. Defects in this granule exocytosis and perforin pathways result in clinical syndrome of HLH, Fig 6. (2) FAS & FASL pathway recruits caspase 10 and finally caspase 3. Caspase is the effector enzyme responsible for apoptosis. (3) Release of Cytochrome C by Mitochondria in response to genotoxic stress due to various stimuli to the cell. Cytochrome C activates caspase 9 to caspase 3, the terminal effector caspase.



Pic. 30: show massive Hepatosplenomegaly & lymphadenopathy in 2 infants with ALPS.

BK; an 8 month old male was admitted with a complaint of intermittent fever for 1 month and an enlarging abdomen. His parents (3rd degree consanguinity) had 4 children. The first, a male, died at 3 days of age; the second, a female is now 9 yrs and healthy; then an abortion at 4 months; the third male died at 7 months of age; BK is the 4 th child, presently 8 months old.

On admission he was afebrile; ht & wt were < 3rd percentile; cervical L.N. was palpable; lungs on auscultation had R sided bronchial breath sounds; liver 5 cms & spleen 8 cms were palpable.

CBC: Hb 7.7 gm/dl, WBC 95,700/mm^3; Poly 11%, Lymphocytes 88%, Eosinophils 1%; platelets 196,000/mm^3; ANC 2045/mm^3; ALC 90,000 /mm^3. Chest X ray showed consolidation in RUL & widening of the mediastinum.

On an ALC of 90,000 LSSA showed that 87% of cells were (DNT) with CD4-, CD8-, TCR $\alpha\beta$+ (Table 7)

WBC remained > 50,000 with almost all lymphocytes, for 3 months, then decreased to 35,500 with an increase in neutrophils, polys > lymphocytes. Also the spleen has regressed 2 cms but there is still quite large. RUL pneumonia & lymphadenopathy are present in the chest & abdomen respectively.

Prognosis in ALPS can be good as the child grows older, his LNs may regress & he may do well.

Table (7): LSSA report of patient BK

Test	No %	Absolute value	Normal range
ALC	88	89884	2800 - 10400
DNT cells CD4-, CD8-, TCR αβ+	87% of TCR αβ+ 86% of CD3 cells		
CD3, HLA DR (activated T Cells)	73	65616	100 – 600 correlates with severity of ALPS

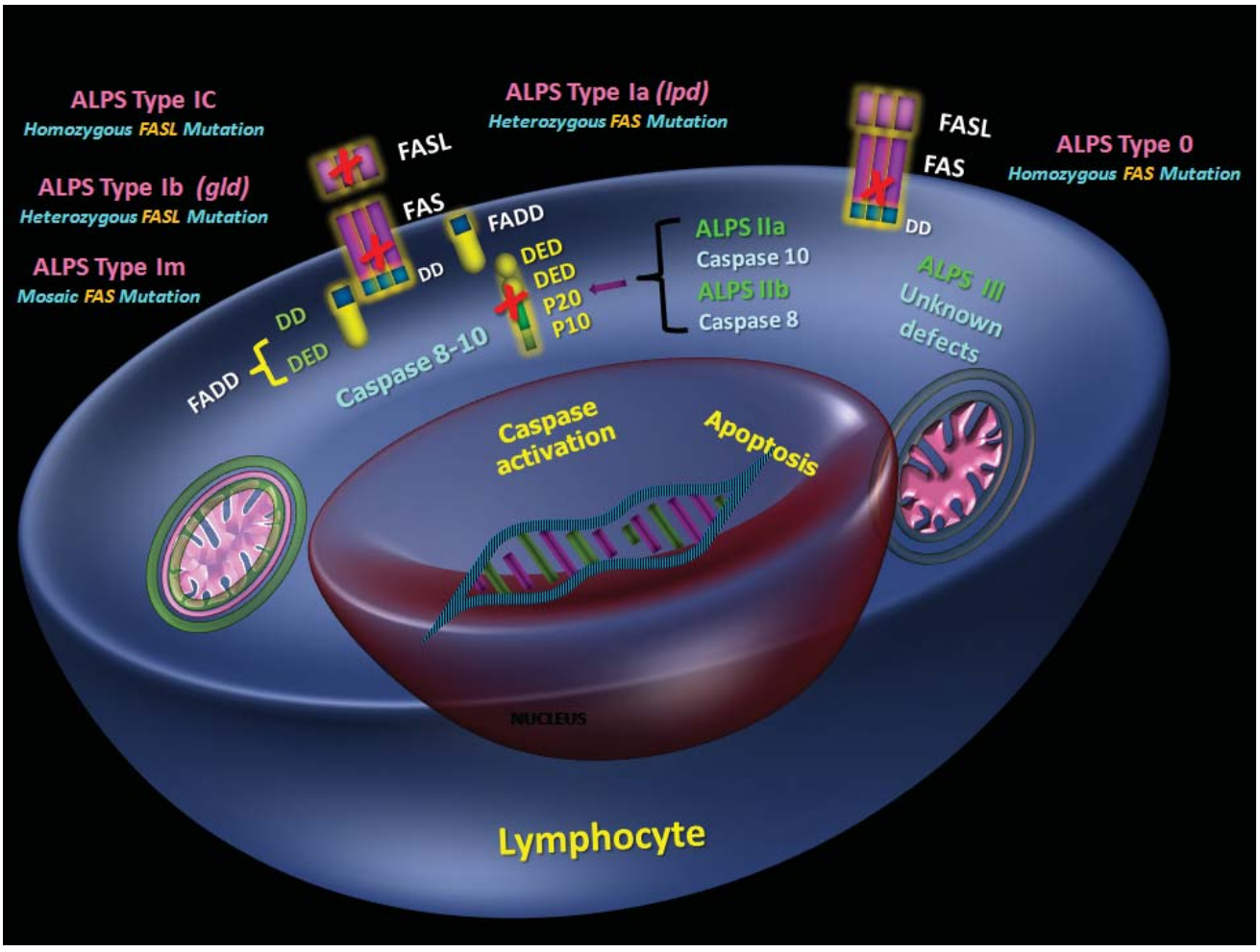


Fig. 22: show current classification of ALPS (Autoimmune Lymphoproliferative Syndrome). ALPS is due to defect in FAS, FASL, or in Caspases 8 & 10. In many cases of ALPS, molecular defects are yet to be characterised (ALPS Type III). ALPS Type Ia (Heterozygous FAS deficiency) is the commonest cause of ALPS. The second most common cause is ALPS Type Im (Mosaic FAS mutation) followed by ALPS Type Ib (Heterozygous FASL mutation). Homozygous mutation in FAS (ALPS Type 0) & FASL (ALPS Type Ic) are very rare. BK, our patient, has Homozygous FASL mutation. This is very uncommon. He presented with a massive Hepatosplenomegaly and extreme lymphocytosis. Both his parents have heterozygous FASL mutation (ALPS Type Ib) but are clinically asymptomatic. ALPS Type IIa (Caspase 10 defect) & ALPS Type IIb (Caspase 8 defect) are rare causes of ALPS.

Patient was admitted for 2nd time on 20.8.09 with intermittent fever for 2 days & diarrhea; HRCT again showed pneumonia. On 7.9.2009 Mycophenolate was started at 600mg/m^2. 3rd admission was on 9.9.2009 with bilious vomiting & abdominal distension; CT scan showed multiple enlarged para aortic Lymph nodes 6 - 7 mm in size. Hb was 6.8 gm/dl, WBC 33,500 / mm^3; ALC 13,400 / mm^3; platelets 276,000 / mm^3, serum lipase 10^9 IU/L, cholesterol 145 mg/dl & triglycerides 182.7 mg/dl. BK was discharged once bilious vomiting settled. He is on regular FU & doing well.

His genetic studies were sent to Dr. Frédérick Rieux-Laucal in Paris & it showed Homozygous FASL mutation. Fig 22 shows current classification of ALPS.

In cases of Evans syndrome, 45% have shown increased DNT cells suggestive of ALPS. Thus always enumerate DNT CD3+, TCR $\alpha\beta$ + cells in all cases of Evan's syndrome.

The Complement System:

Activated by (1) Classical pathway in which the C1q,r,s proteins interact with Abs on the bacterial surfaces of S. pneumonia, H. influenzae & N. meningococcus & lyse them. (2) Lectin pathway in which mannose binding protein deposits on bacteria & (3) Alternative pathway in which Factor B amplifies the response to bacterial infections.

Complement defects result in (1) Pyogenic infections & (2) Autoimmune diseases: e.g. Systemic lupus erythematosus (SLE). SLE develops in 93% of C1q deficiency patients. We have a patient with a familial Hemolytic uremic syndrome (HUS) who did not have a preceding diarrhea & that implies a Factor H, I or MCP (Membrane co factor protein) deficiency (alternative pathway).

Table 9: Depicts common complement deficiency & their clinical presentation:

Complement def.	Autoimmunity	Infections	Other
C1 q deficiency	93% develop SLE	30% get significant bacterial infections	10% die
C2	SLE	Encapsulated organisms.	C2 is the most common inherited deficiency; gene is on chromosome 6 within the MHC.
C3	SLE also	Infection	
C4 Rare, (2 genes, 4 alleles,C4A & C4B)	Early onset of SLE, Henoch-Schonlein, purpura, ITP, celiac disease	Bacterial infections are common & can be severe.	
Factor P (Properdin)		Risk factor for meningococcal infections; upto 50% of patients suffer from meningococcal infections.	
C5-C9		Increased susceptibility to gram-ve bacteria, especially Nisseria	

Complement system can destroy self tissues if self activated hence nature has provided inhibitors. The inhibitors of complement system are C1 inhibitor, factor H, Factor I, MCP (membrane cofactor protein) and DAF (Decay Accelerating Factor) which along with CD 59 is defective in PNH.

Table 10: Depicts common complement Inhibitor deficiency & their clinical presentation:

Complement inhibitor deficiency	Clinical presentation	
C1 inhibitor	Hereditary angioedema. Sub mucosal / SC edema	
Factor I Deficiency		Infections with encapsulated organisms.
Factor H & MCP	deficiency cause familial HUS which is usually D-ve unlike in HUS in non familial cases	

Conclusions:

With such classical text books on PID (by Ochs Smith & Puck) available, was it necessary to write a booklet on PID in Mumbai? The answer is definitely yes.

PIDs at BJWHC manifest themselves frequently in the first 2 yrs of life. PIDs are genetic, inheritable disorders. In Mumbai along with the known factors of overcrowding and an unhygienic environment, non immunodeficient babies also suffer from repeated or unusual infections at very early ages and differential diagnosis can become very difficult. We cannot justifiably order extensive immunodeficiency tests, and we strongly recommend the pediatrician be patient and watchful for other signs and symptoms of a PID. Also, it is better to do immune tests when the child is stable, or better, or recovered & well, before making a “most probable diagnosis” of PID.

In ICU, a baby with sepsis, an elevated PMN count and a relatively decreased ALC, was wrongly diagnosed as SCID on LSSA. When he recovered he had no evidence of SCID on retesting! We have attempted to write this booklet so that the reader may look at an infant from several points of view. Time is our best ally; do not be hasty in your judgement. We feel that history of the present illness; consanguinous parents, male sex, illness, death in other sibs; weight, physical examination inclusive of examining the mouth & anus, Lymph nodes, Hepatosplenomegaly, and skin; finally CBC and ALC should provide help in suspecting a PID. The other lab test of importance is Microbiology- what organism was isolated from the child, was it a bacteria, virus, fungii or a parasite?

We are looking forward to having a cytogenetic division, establishing a stem cell transplantation and establishing a strong molecular lab.

We have discussed 4 of our common problems in PIDs in detail and we hope in future, to extend our work to other PIDs to complete the list to date, & to include therapeutic aspects of PIDs and the advances that are being made in the understanding of PIDs, and advice regarding prevention of PIDs. We have also realized the impact of immune deficiency on our own thinking and the way to make a diagnosis of PID.

For a comprehensive care for PIDs we have a strategic liaison with NIIH (National Institute of Immunohematology) for establishing immune tests, & TATA Memorial Centre (TMC) for tissue sections &immune markers on the tissue specimens. We are fortunate in already having subspecialty services in our children's hospital. We urgently need to expand to performing cytogenetic tests for PIDs & establishing molecular techniques for detection of carriers and providing antenatal diagnosis to prevent the birth of children with life threatening PIDs. This is the time for collaboration and cooperation, and PID is one area which has brought BJWHC, NIIH & TMC together.

Acknowledgements & Credits

BJWHC: Dean Dr Uma Ali, CSE Dr S. B. Chavan, Prof. Dr Shakuntala Prabhu.

NIIH: Dr K Ghosh, Dr Manisha Madkaikar

TMC: Dr Sumeet Gujral; Dr Mani Subramaniam

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Dr M R Lokeshwar.

Dr. Alain Fischer, Dr. J.L. Casanova, Dr. Bobby Gaspar, Dr. S. Kaveri, Dr. Paul Veys, D. S. Kumararatne and others.

All our referring doctors

All our patients

Credits:

Dr Zinet Currimbhoy has contributed to the manuscript of the book.

DR Mukesh Desai has contributed to the figures, photomicrographs & pictures in the book.

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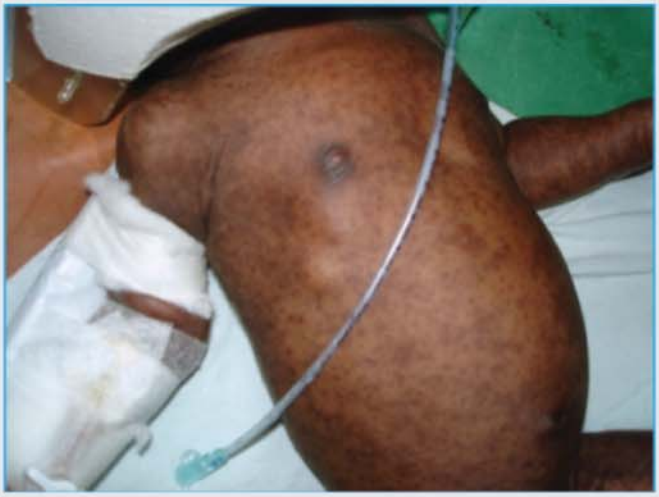
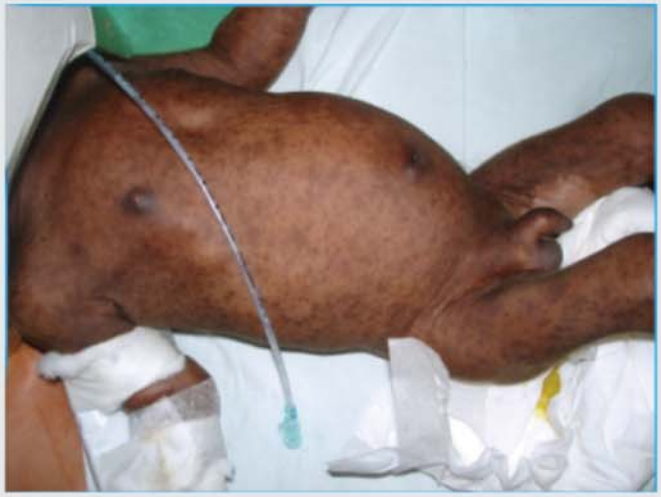
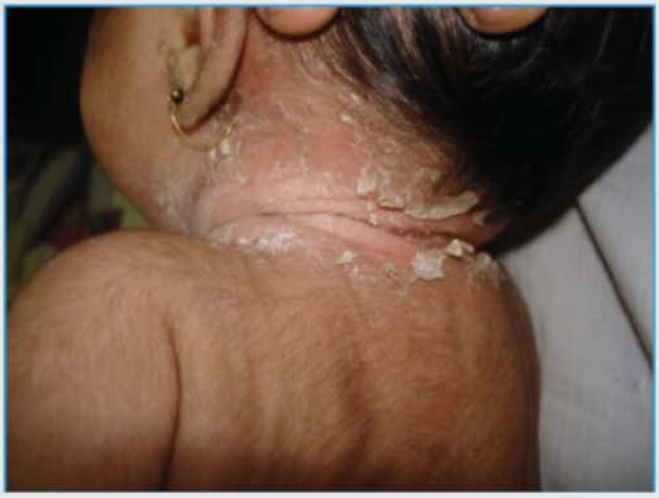
Precautions in PIDs

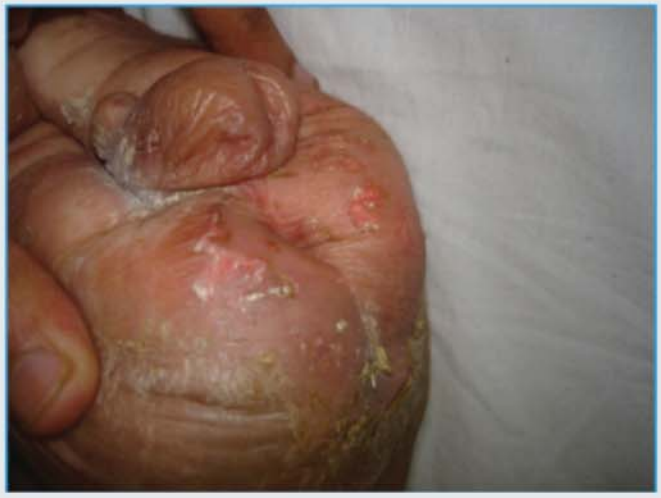
- Do not Immunize with live vaccines: Death or severe consequences can result.
- Do not Transfuse any Blood or Blood product without irradiation to prevent TAGvHD





Pic show 2 cases of SCID with GvHD (Graft vs Host Disease) due to maternally transmitted Lymphocytes & Tx.





Hence, Irradiate all Blood & Blood Product before Tx In any suspected case of PID.