Immunodeficiency II

26.10.2012

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- Cellular primary immunodeficiencies
- Secondary immunodeficiencis
- Immunological laboratory tests
- Case studies

1. Combined T and B deficiencies

SCID

severe **c**ombined **i**mmuno**d**eficiency

- heterogeneous group of rare geneticaly determined disorders resulting from impaired T and B functions
- 1-5 per 500 000 live births (in CR: 1 new SCID /2-4 years)

- (SCID might be a cause of some undiagnosed infant death)

Classification of SCID

- **T-B-NK-SCID**
  - reticular dysgenesis*; defect of ADA genes* →
  → accumulation of toxic purine metabolites in lymphocytes
- **T-B-NK+**
  - (*RAG1/2 defects* →defects of TCR,BCR rearrangement)
- **T-B+NK-**
  - (approximately 50 % of SCID are *X-linked defect of IL-2 receptor γ chain*)

..

Characteristic features of SCID

- **Onset of infections in the first weeks/months** of life
- often **viral, fungal** (candida) infections (rather than bacterial)
- **chronic diarrhoea**
- respiratory infections **(Pneumocystis jirovecii**)
- **oral thrush**, **ecsema** - erytrodermia
- absence of obvious infections
- **lymphopenia**
- **serious adverse reaction** to tbc vaccination

Some other CID

- **Di George syndrome**

  thymus hypoplazia or aplazia; abnormal facies, hypoparathyroidism, cardiovascular defects; immunological abnormalities (variable severity)

- **Chronic mucocutaneous candidiasis**

  Candida albicans infections, endocrine abnormalities (hypothyroidism, Addison´s d., recurrent bacterial infections) e.g. *AIRE gen mutation*

- **Wiskott-Aldrich syndrome**

  thrombocytopenia, eczema, recurrent infections, malignant diseases *(WASP gen mutation)*

Management of patients with defects of cellular immunity

- Early recognition of PID (differentiation from HIV)
- Avoidance of infections: antimicrobial prophylaxis
- Avoidance of live vaccines, conventional blood transfusions (? Why ?)
- Grafting of viable immunocompetent cells (bone marrow transplantation; stem cell transplantation)
- Replacement of missing factors (short time effect)

2.

Secondary immunodeficiencies

Secondary ID

- Far more common than primary ID
- There are a lot of causes which can lead to
  - **failure of synthesis** of the immune components (quantitative/ qualitative)

or

  - to **intensive consumption**, catabolism, loss of the immune components

Clinical symptoms of secondary ID

- **Susceptibility to infection, malignancy**…

  (severity of ID depends on the cause)
- ! **More complex disorder of the immune system than PID**
- An isolated deffect of one part of the immune system is very rare
- Defficiency of innate immunity is more common

Main causes of secondary ID

- **Metabolic diseases** (DM), **malnutrition**, avitaminosis, **bowel diseases** (infections or IBD), **tumours**…
- **Failure of innate barriers** (burns, dermatitis, toxic lesions, injuries…)
- **Infection** – HIV;CMV; influenza, measles, malaria…
- **Lymphoproliferative diseases**
- **Autoimmune diseases**
- **Iatrogenic disorders** (operation, drugs, radiation…)

- Malignancy and ID

- **Pathophysiology of ID**

1. Role of the immune system:
   - antibodies or T cells (to destroy tumour cells) cross-react with normal tissues and destroy them
2. Role of substances produced by tumours
   (hormones, enzymes, cytokines….)

Paraneoplastic syndrome

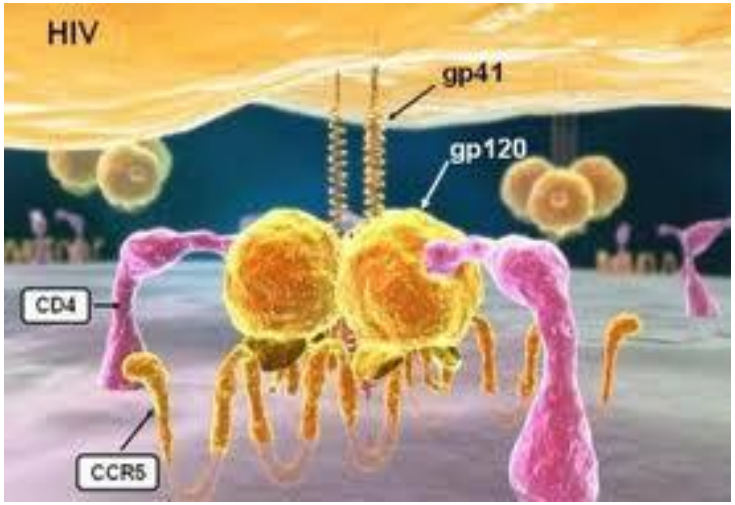
- Involves symptoms resulting from substances produced by tumours and/or from activity of the immune system

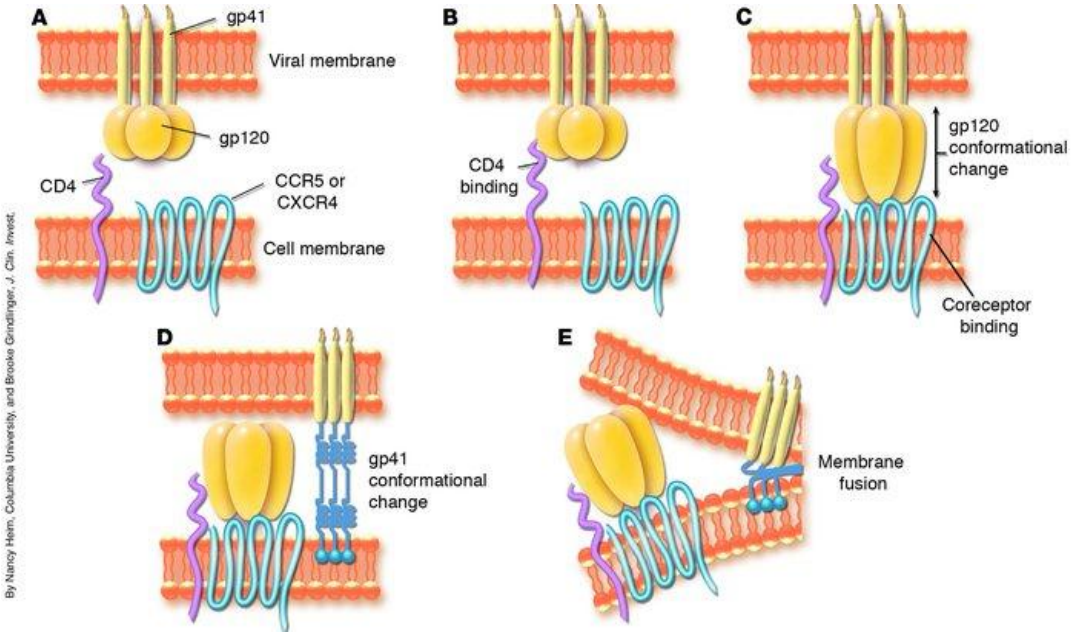
- Infection and secondary ID

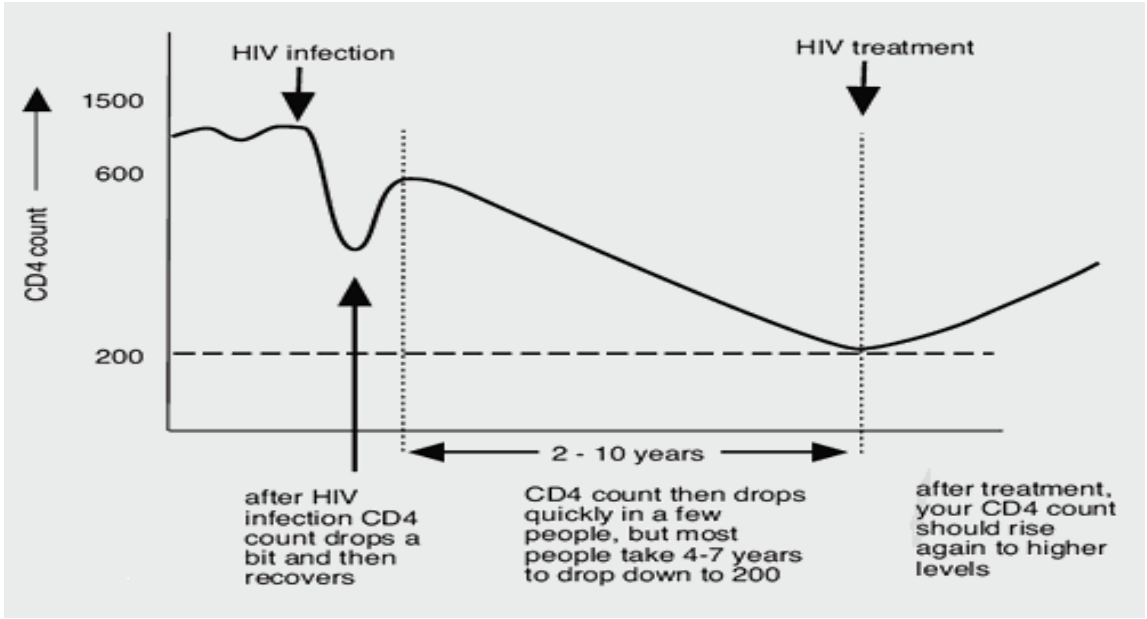
HIV infection: spectrum of disorders

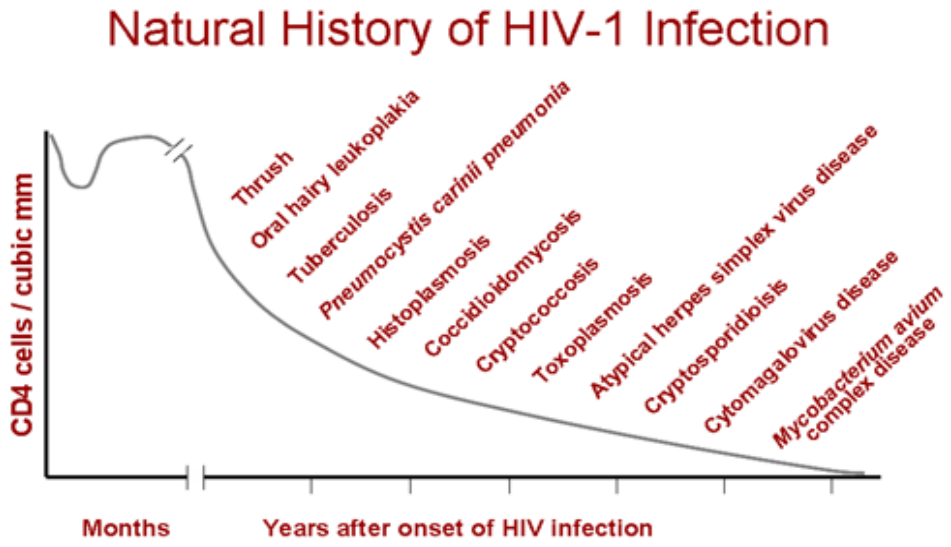
- Acute glandular fever-like illness

- **AIDS**-a final stage of HIV infection-
  - Severe opportunistic infections
  - Tumours
  - Dementia (due to brain atrophy)
  - Autoimmune diseases


HIV
gp41
gp120
CD4
CCR5


A
gp41
Viral membrane
gp120
CD4
CCR5 or
CXCR4
Cell membrane
B
CD4
binding
C
gp120
conformational
change
Coreceptor
binding
D
gp41
conformational
change
E
Membrane
fusion
By Nancy Hem, Columbia University, and Brooke Grindlinger. J. Clin. Invest.


HIV infection
HIV treatment
1500
600
200
CD4 count
2 - 10 years
after HIV infection CD4 count drops a bit and then recovers
CD4 count then drops quickly in a few people, but most people take 4-7 years to drop down to 200
after treatment, your CD4 count should rise again to higher levels


Natural History of HIV-1 Infection
CD4 cells / cubic mm
Thrush
Oral hairy leukoplakia
Tuberculosis
Pneumocystis carinii pneumonia
Histoplasmosis
Coccidioidomycosis
Cryptococcosis
Toxoplasmosis
Atypical herpes simplex virus disease
Cryptosporidiosis
Cytomagalovirus disease
Mycobacterium avium complex disease
Months
Years after onset of HIV infection

CD 4+ count (cells/µl) / type of infection

1200 – 700 **normal healthy adults**

<500 tuberculosis (PTB), oral or vaginal thrush, herpes zoster, herpes simplex virus, non-Hodgkin´s lymphoma

<300 **very severe** thrush (candidal infec.) & oral hairy leukoplakia,

<200 cryptococcal meningoencephalitis, *Pneumocystis carinii* pneumonia, *Candida albicans* esophagitis

<100 toxoplasmic encephalitis+ CHORIORETINITIS, cryptococcal meningitis, AIDS dementia, Progressive multifocal leukoencephalopathy(JC virus), wasting syndrome - extreme weight loss and anorexia caused by HIV

<50 CMV retinitis, Mycobacterium avium (MAC) infection

Causes of CD4+ lymphopenia

Secondary

- HIV infection (AIDS)
- Autoimmune (antilymphocytic antibodies)
- Malignancy

Primary, idiopathic

  - Clinical presentation: recurrent papillomavirus infection

Splenectomy /immunodeficiency state

- Defects of phagocytosis
- Lower IgM level
- Susceptibility to infection (sepsis) caused by encapsulated bacteria

Immunization after splenectomy:

- ***Streptococcus pneumoniae***
- ***Haemophilus influenzae type b***
- ***Neisseria meningitidis***

Immunological tests

Evaluation of humoral immunity

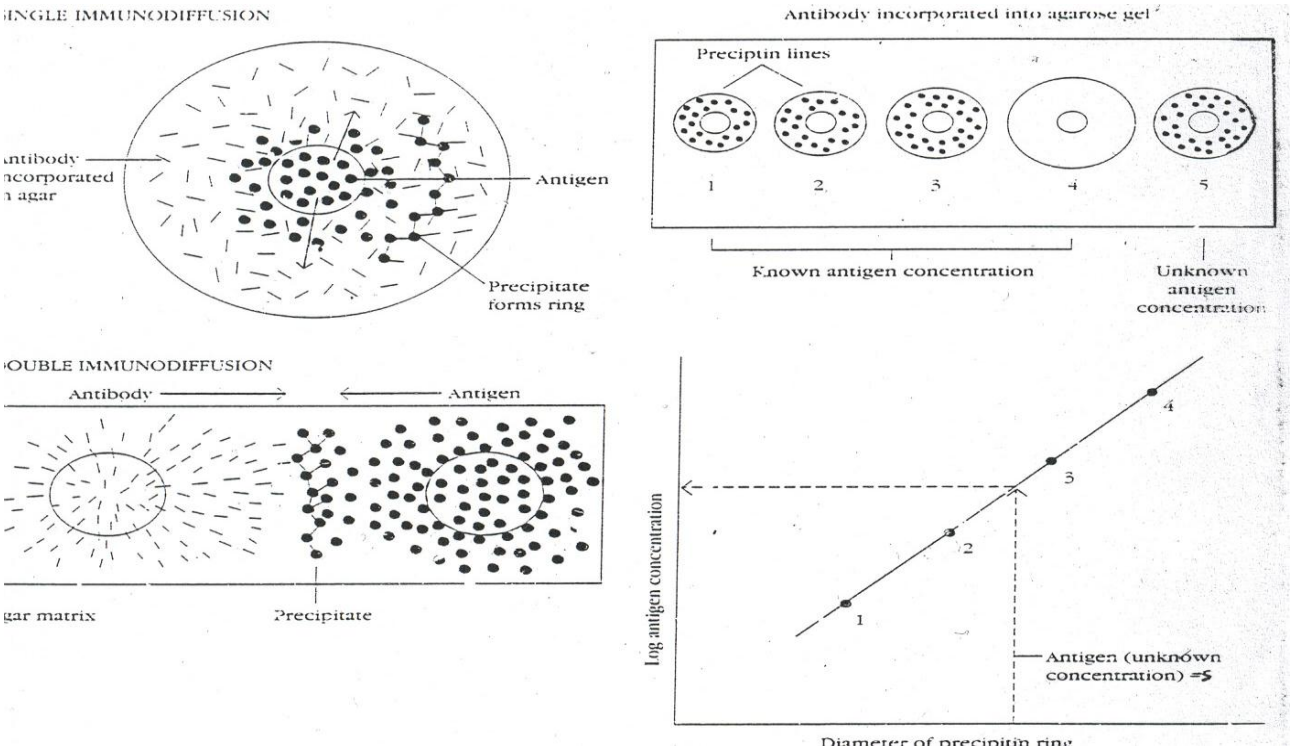
- Serum immunoglobulin levels (Ig classes, subclasses)
- Complement evaluation
- Specific antibody levels to vaccination antigens (tetanus + diphteria toxoid, polysaccharide antigen (STP)

Evaluation of cell-mediated immunity

- Leukocyte (and lymphocyte) number
- T and B lymphocyte enumeration (CD4, CD8, CD19)
- Test of T cell function – stimulation with mitogens
- Phagocytosis (activity)
- Skin test (for delayed type hypersenzitivity)

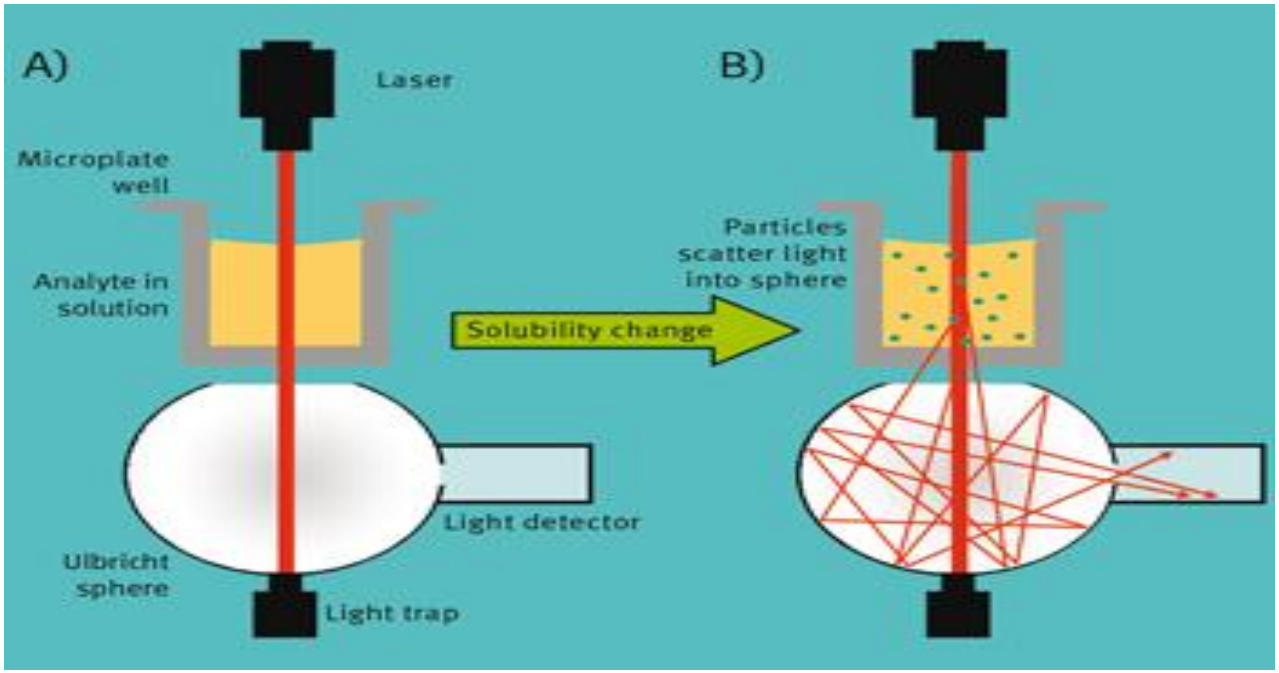
1.Serum level of immunoglobulins

- *a/ immunoprecipitation - radial immunodiffusion (Mancini´s)*



- b/ **Current tests (!):**

Turbidimetry and **nephelometry**



Medium with dispersed particles (different refractive indices)

Attenuated light intensity by scaterring on particles

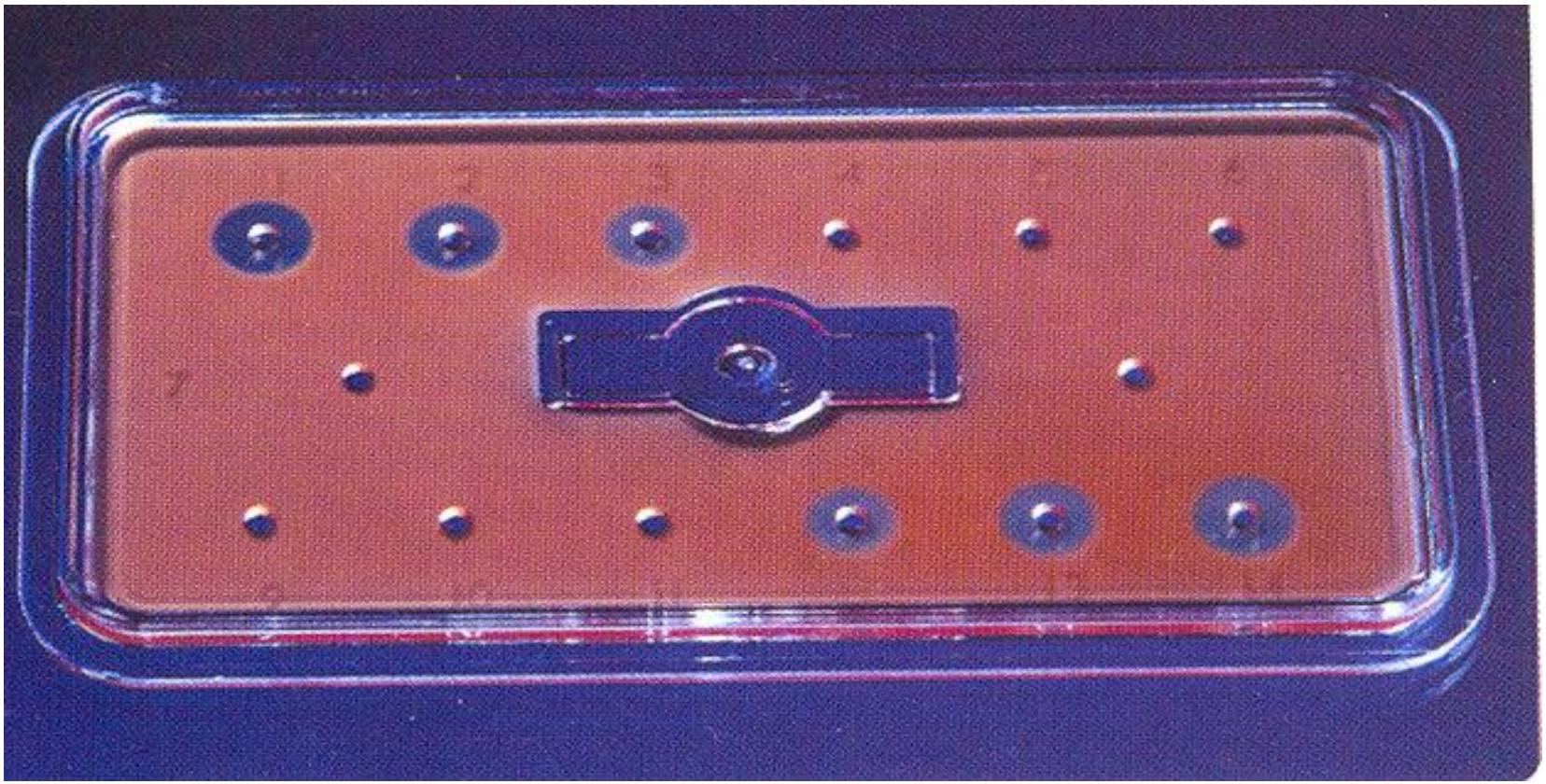
2. Complement evaluation

- **a/ Quantitative analysis: levels of complement components**
  - (C3, C4, C1q, C1 inh, MBL…)
  - Test: nephelometry, immunoprecipitation

- **b/ Functional test:**
  - Hemolytic activity of the complement (CH100, CH50, AP 50)

Hemolytic activity of complement (CH 100 or CH50 test)

(in agar, or in tube)



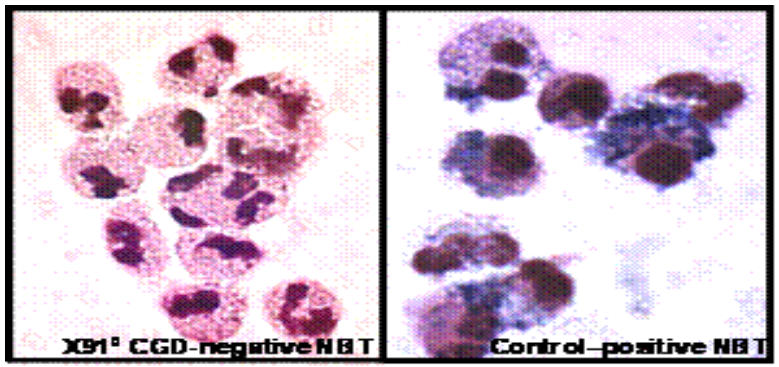
3. Phagocytosis evaluation

- A/ **Quantitative determination of leukocyte phagocytosis**
  - Microscopic test with hydrophilic particles

    or with fluorescein-labelled opsonized bacteria (ESC-FITC)
  - **Result:** overall percentage of monocytes and neutrophils ingesting one or more bacteria per cell
  - Indication: neutrophil dysfunctions, immunosupressive treatment

B/ Testing of bactericidal mechanisms

test of the respiratory burst (generation of superoxide ions)
methods: flow cytometric test (dihydrorhodamine rhodamine) or NBT



NBT reduction test

NBT

Phagocytic cells can change a colourless chemical called nitro blue tetrazolium (NBT**) into a deep blue colour**. This change is mediated by generated oxygen.

4. Lymphocyte subpopulations

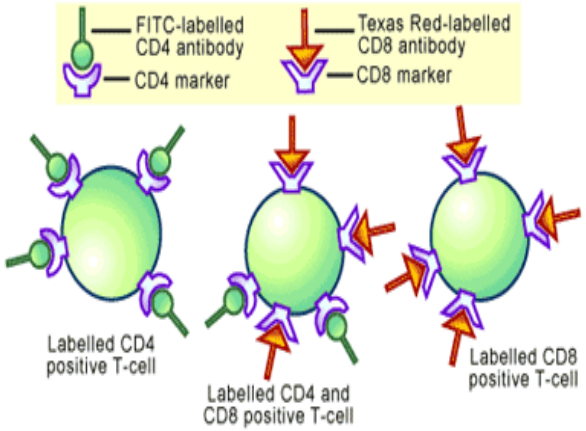
Flow cytometry identifies cell subsets
on basis of

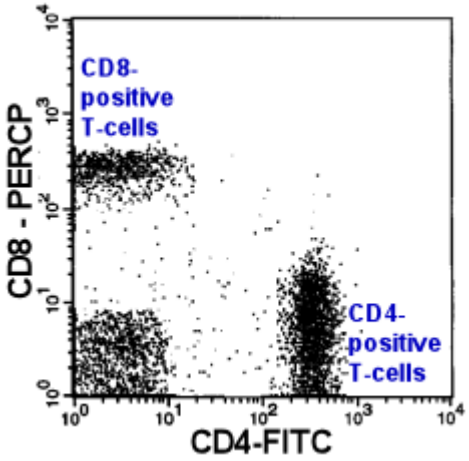
- **physical characteristics** (size, granularity)
- **abilities to bind fluorochrome tagged monoclonal antibodies** (against surface molecules or intracellular ones)

- *a lineage - and differentiation-specific cell molecules*
- *a monoclonal antibodies specific for the molecule*
- *different types of fluorochromes*

Fluorescent flow cytometry analysis

A specific example: the analysis of the markers on T-cells





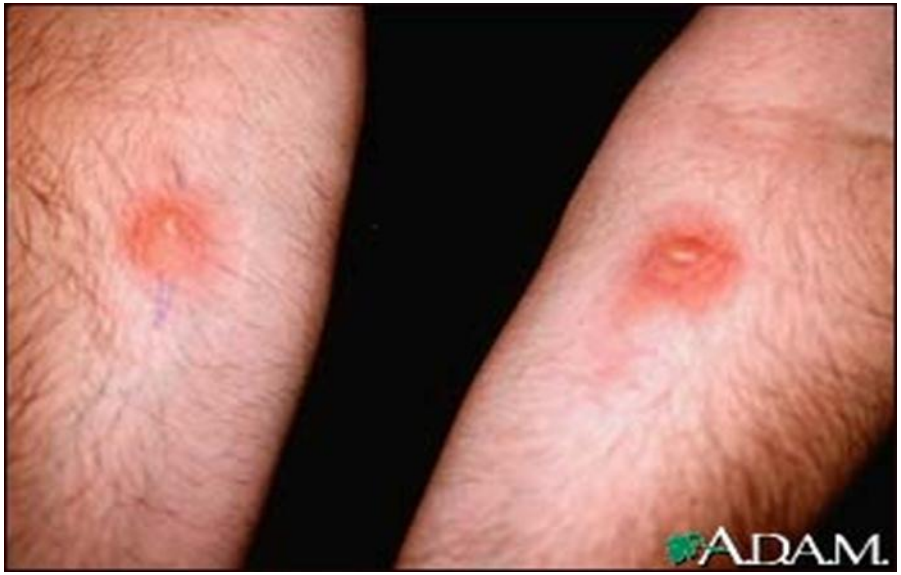
5. Lymphocyte proliferation test

- Measures ability of lymphocytes to proliferate in response to various stimuli such as
  - plant lectins: pokeweed mitogen (PWM)
    phytohaemagglutinin (PHA)
  - bacterial lipopolysaccharide (LPS), candida

  On basis of ^{3}H thymidine incorporation

  in DNA strands
- Result: counted radioactivity (cpm – count per minute)

6. Skin testing of DTH



Candida albicans and PPD test

4 Stages of Testing for Primary Immunodeficiency

1
- History and physical examination, height and weight
- CBC and differential
- Quantitative Immunoglobulin levels IgG, IgM, IgA (related to age)

2
- Specific antibody responses (tetanus, diphtheria)
- Response to pneumococcal vaccine (pre/post) (for ages 3 and up)
- IgG subclass analysis

3
- Candida and Tetanus skin tests
- Lymphocyte surface markers CD3/CD4/CD8/CD19/CD16/CD56
- Mononuclear lymphocyte proliferation studies (using mitogen and antigen stimulation)
- Neutrophil oxidation burst (if indicated)

4
- Complement screening CH50, C3, C4
- Enzyme measurements (adenosine deaminase, purine nucleoside phosphorylase)
- Phagocyte studies (surface glycoproteins, mobility, phagocytosis)
- NK cytotoxicity studies
- Further complement studies AH50
- Neo antigen to test antibody production
- Other surface/cytoplasmic molecules
- Cytokine receptor studies
- Family/genetic studies

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National Heart, Lung, and Blood Institute (NHLBI)

National Cancer Institute

National Institute of Allergy and Infectious Diseases (NIAID)

National Institute of Child Health and Human Development (NICHD)

Baxter BioScience

CSL Behring Biotherapies for Life™

GRIFOLS

octapharma

PPTA Plasma Protein Therapeutics Association

Talecris Biotherapeutics

These recommended immunologic tests reflect a consensus of the Jeffrey Modell Foundation Medical Advisory Board. Consultation with Primary Immunodeficiency experts is strongly suggested.

For information or referrals, contact the Jeffrey Modell Foundation: 866-INFO-4-PI | **info4pi.org**

Treatment of ID

(Depends on the type of ID (PID /secondary + humoral/cellular) and severity of defects)

(Treatment of the underlying disease)

Replacement and antimicrobial therapy

- Immunoglobulin replacement therapy (i.v., s.c.; D: 400-600 mg/kg/month) – antibody deficiency
- Hyperimmune Ig (VZV, CMV, HBV…)
- Antibacterial, antiviral, antifungal or antiparasitic therapy or prophylaxis

Stem cell transplantation

(SCID, severe phagocytic disorders)

Gene therapy

(monogenic ID – Bruton´s, X-SCID)

Other methods of treatment of ID patients

- Nutrition – protein rich diet (in developing countries, old people, …)
- Vitamins (vit. C)
- Physical activity
- Hardening, psychotherapy…

- Vit. D ?? (conclucions of study - 2012)
  - Monthly vitamin D supplementation at 100,000 IU over 18 months is not associated with a reduced rate of URTIs.
  - Monthly supplementation with 100,000 IU of vitamin D is not associated with reduced duration or severity of URTIs or missed days from URTIs