Imunodeficiencies

Department of Immunology

November, 2008

Immune system and its function

- benefit x damage
- defense
- immune surveillance
- tolerance

Immune system and its function

- repeated infections → immunodeficiencies
- pathological reaction to environmental antigens → allergy
- pathological reaction to internal antigens → autoimmunity
- defects in immune → tumours

Immune system and its function

- repeated infections → immunodeficiencies
- pathological reaction to environmental antigens → allergy
- pathological reaction to internal antigens → autoimmunity
- defects in immune → tumours

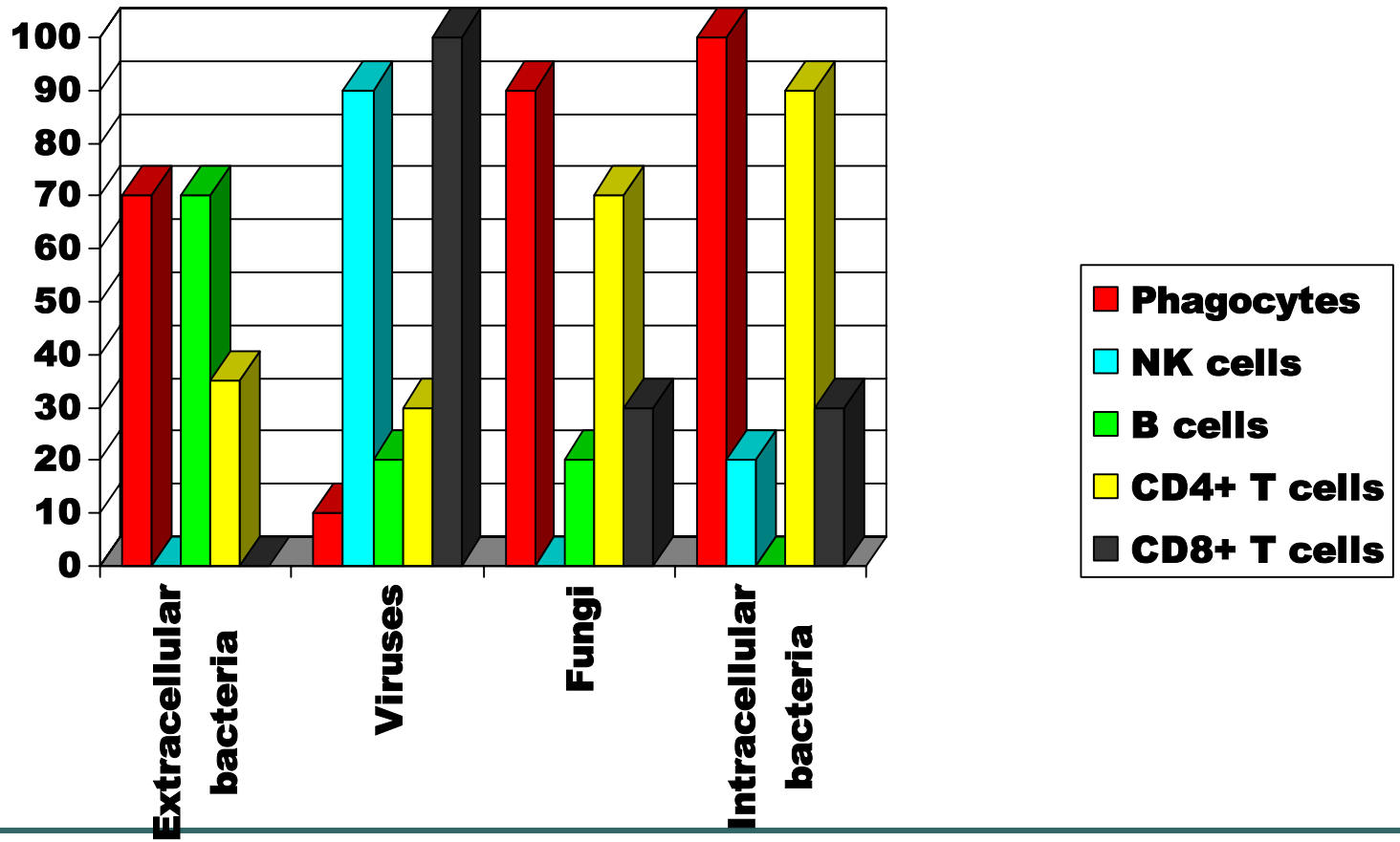
Common defense mechanisms

- skin and mucosa
- cilia, mucus
- hydrochloric acid
- flow of urine
- tears

Roles of immune system components

		defense against
innate immunity		
• humoral - complement	→	• bacteria, some viruses
• cellular - phagocytes, NK cells	→	• bacteria, fungi
adaptive immunity		
• humoral - antibodies	→	• extracelullar bacteria • viruses
• cellular - T lymphocytes	→	• intracelullar bacteria • viruses, fungi

Role of immune system in infection combat



Immunodeficiencies

- 1. primary
  - innate diseases
  - genes coding for immune system components
- 2. secondary
  - secondary immune disorders based on primary cause

Secondary immunodeficiency

= presence of underlying disease

- malignancy (malignancy)
- infection (e.g. HIV)
- malnutrition
- immunosuppresive drugs

Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch

- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway

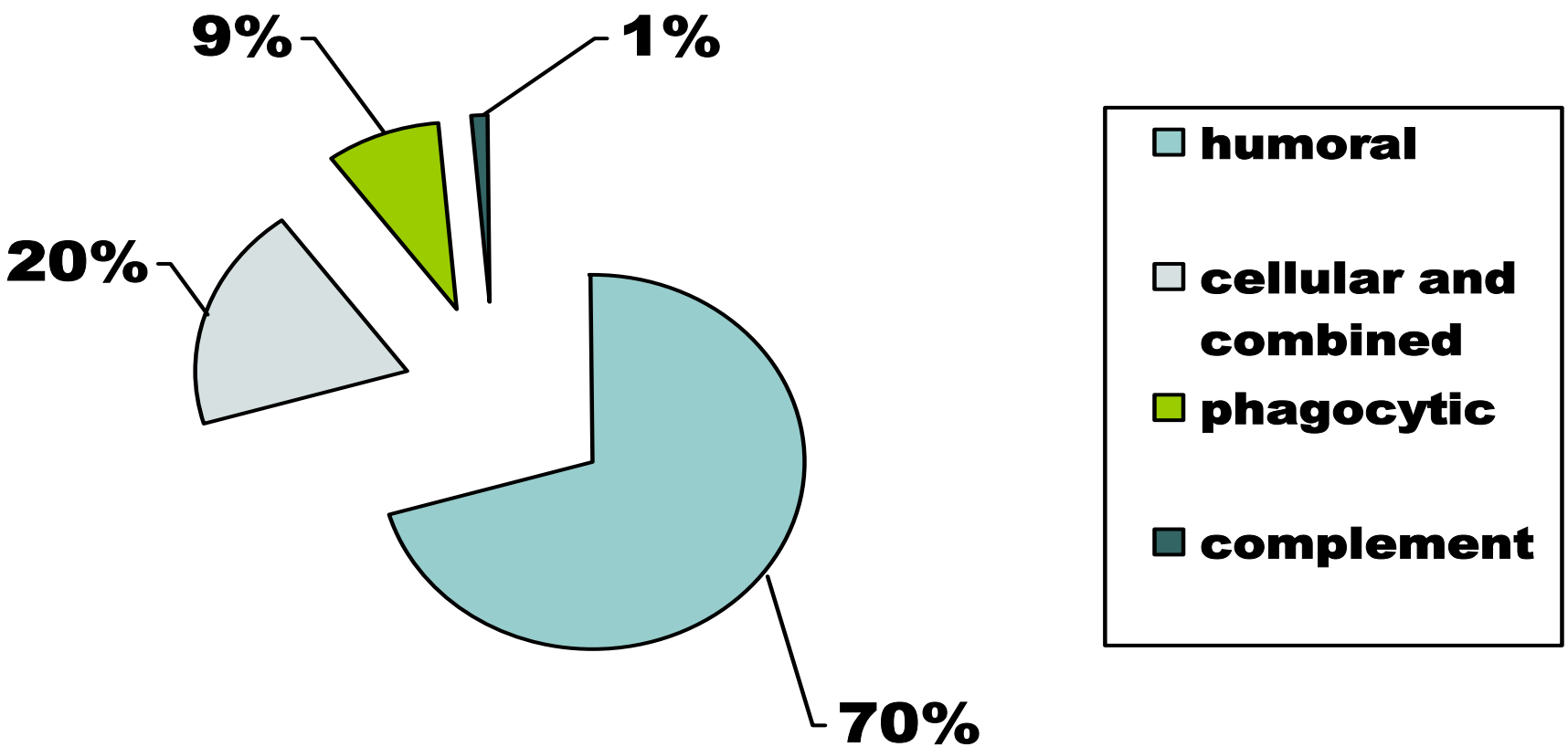
- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)

- Complement
  - particular components
  - regulatory factors

- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis

- Syndromes with compromised DNA repair

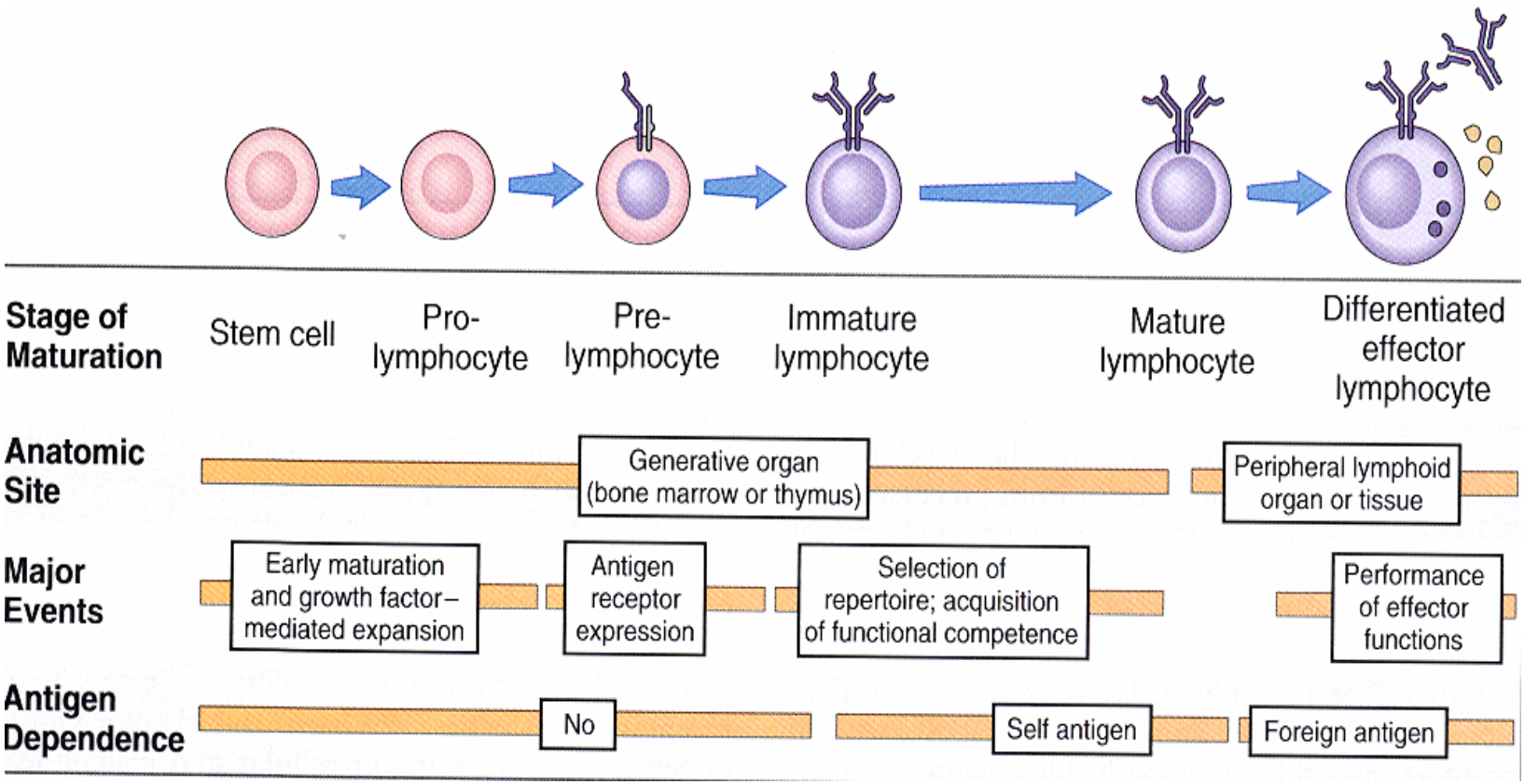
Prevalence of primary immunodeficiencies (PID)



Incidence of PID (examples)

sIgAD	**1 : 500 - 700**
DGS	1 : 4.000 (live births)
CVID	1 : 10.000 – 50.000
SCID	1 : 100.000
CD19 deficit	only a few cases

Differentiation of B and T cells



Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch

- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway

- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)

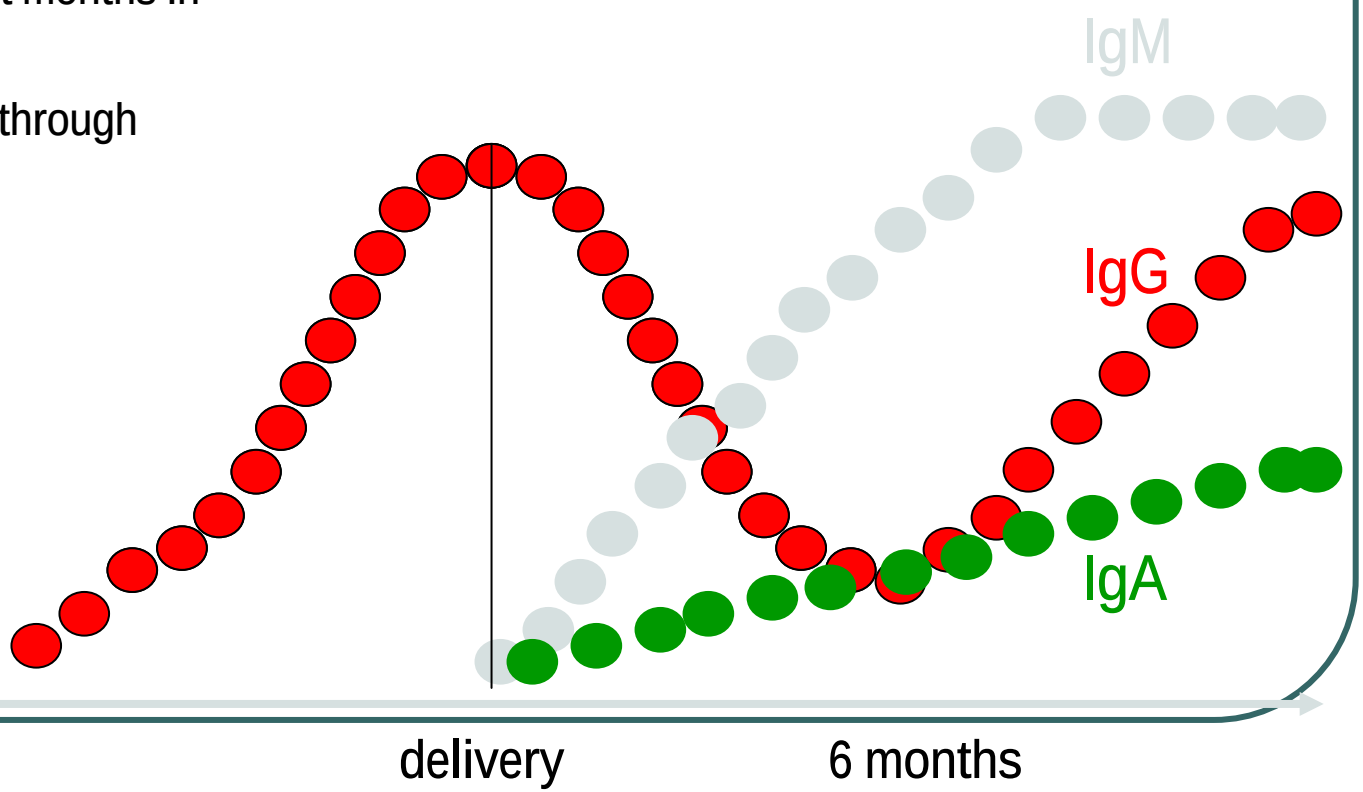
- Complement
  - particular components
  - regulatory factors

- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis

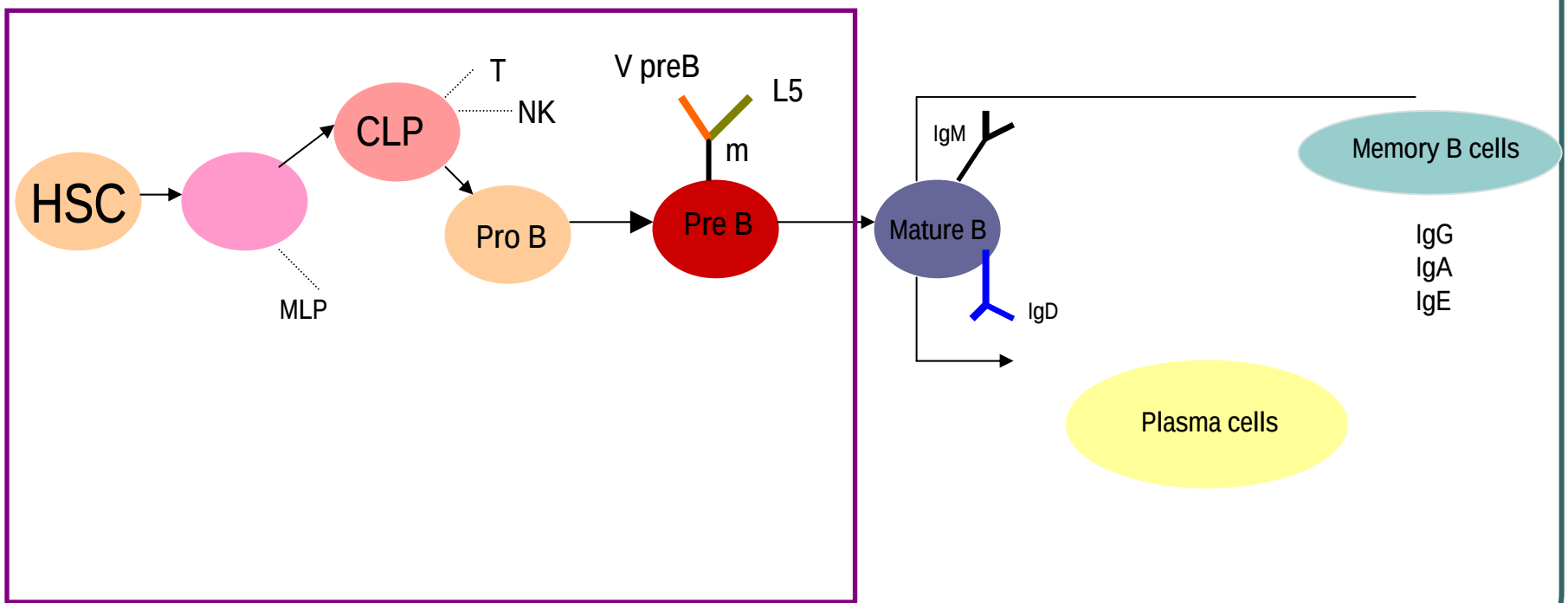
- Syndromes with compromised DNA repair

Levels of antibodies in kids

- production of Ab associated with cellur development of immune-competent cells (starts during 1st months in utero)
- active transport through placenta

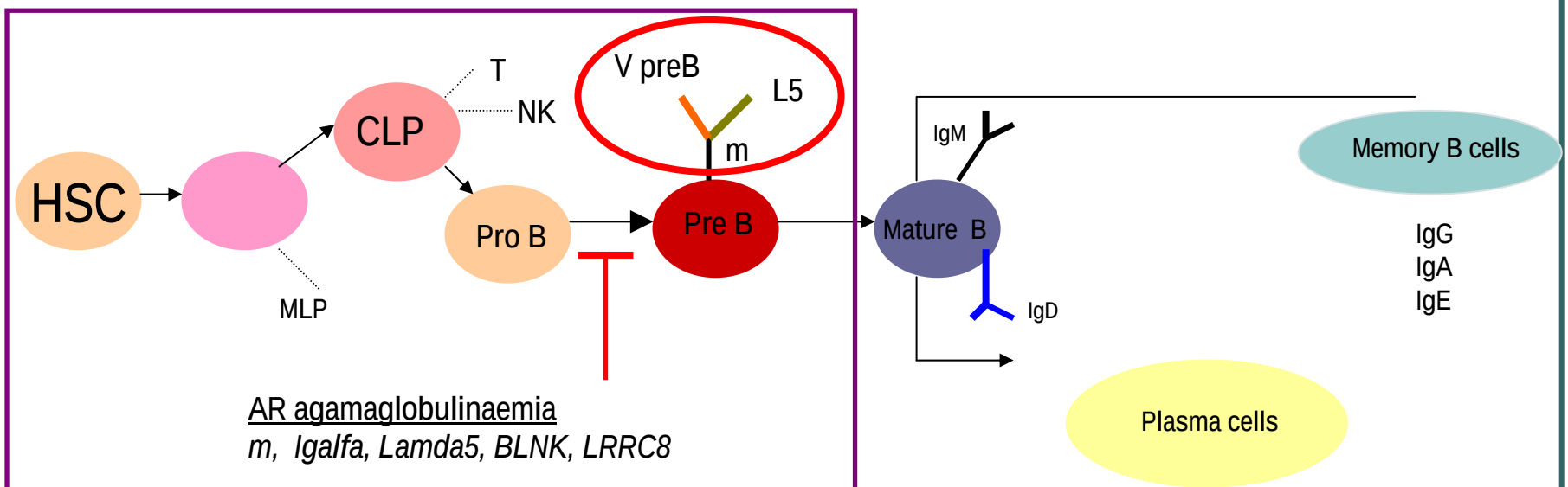


B cell development



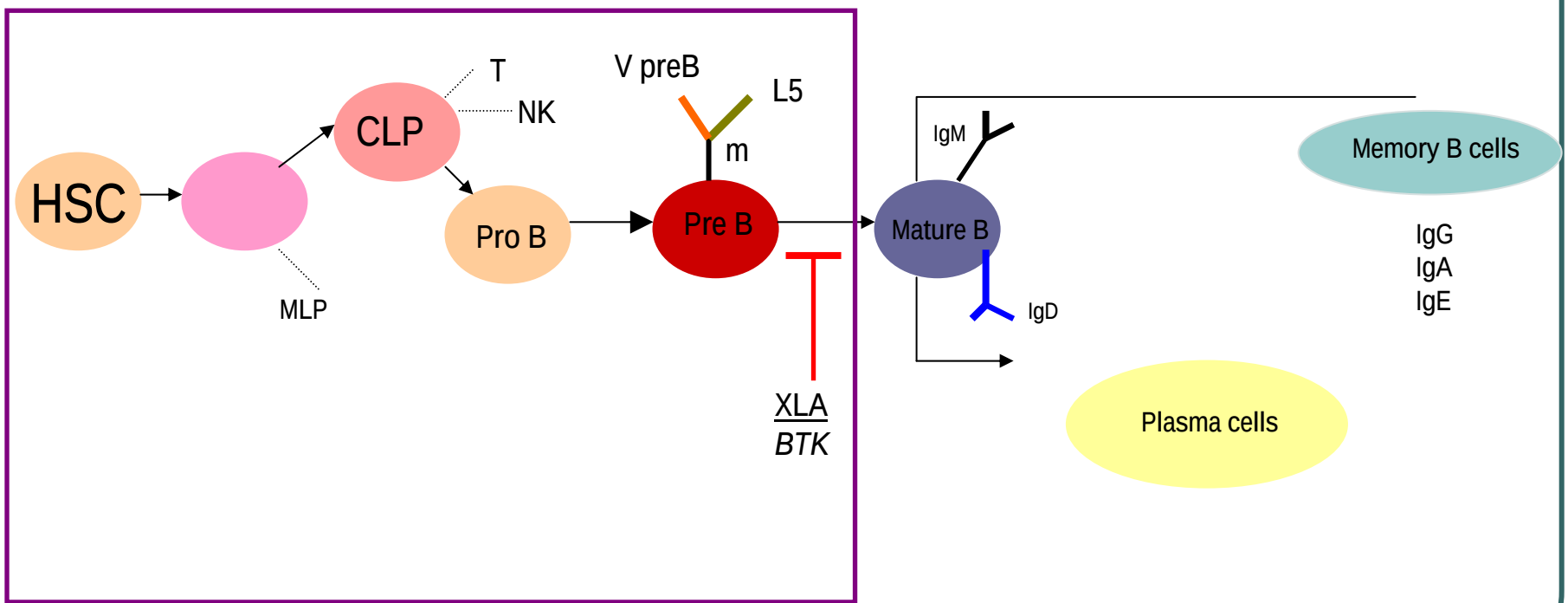
Fischer, Nature Immunology 2004

Agammaglobulinaemia - AR



Fischer, Nature Immunology 2004

Agamaglobulinaemia - XL

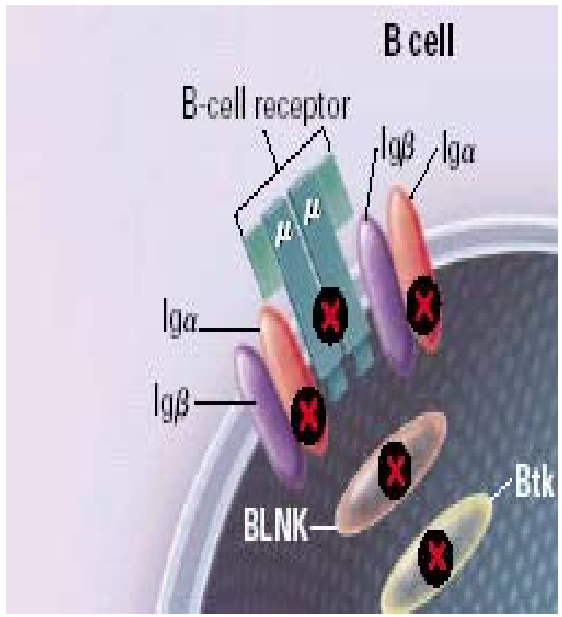


Fischer, Nature Immunology 2004

Bruton agamaglobulinaemia (XLA)

- X-linked agammaglobulinemia
- single gene defect
- lack of B cells

x



- autosomal recessive agammaglobulinaemias (20%)

VAŠEK, 3 y-o boy

VAŠEK Personal history

- IVF, twin A, brother healthy
- perinatal history unremarkable
- pneumonia 3 wks before diagnosis
- regulary vaccinated
- admitted for laryngitis, septic state followed

VAŠEK Laboratory investigation

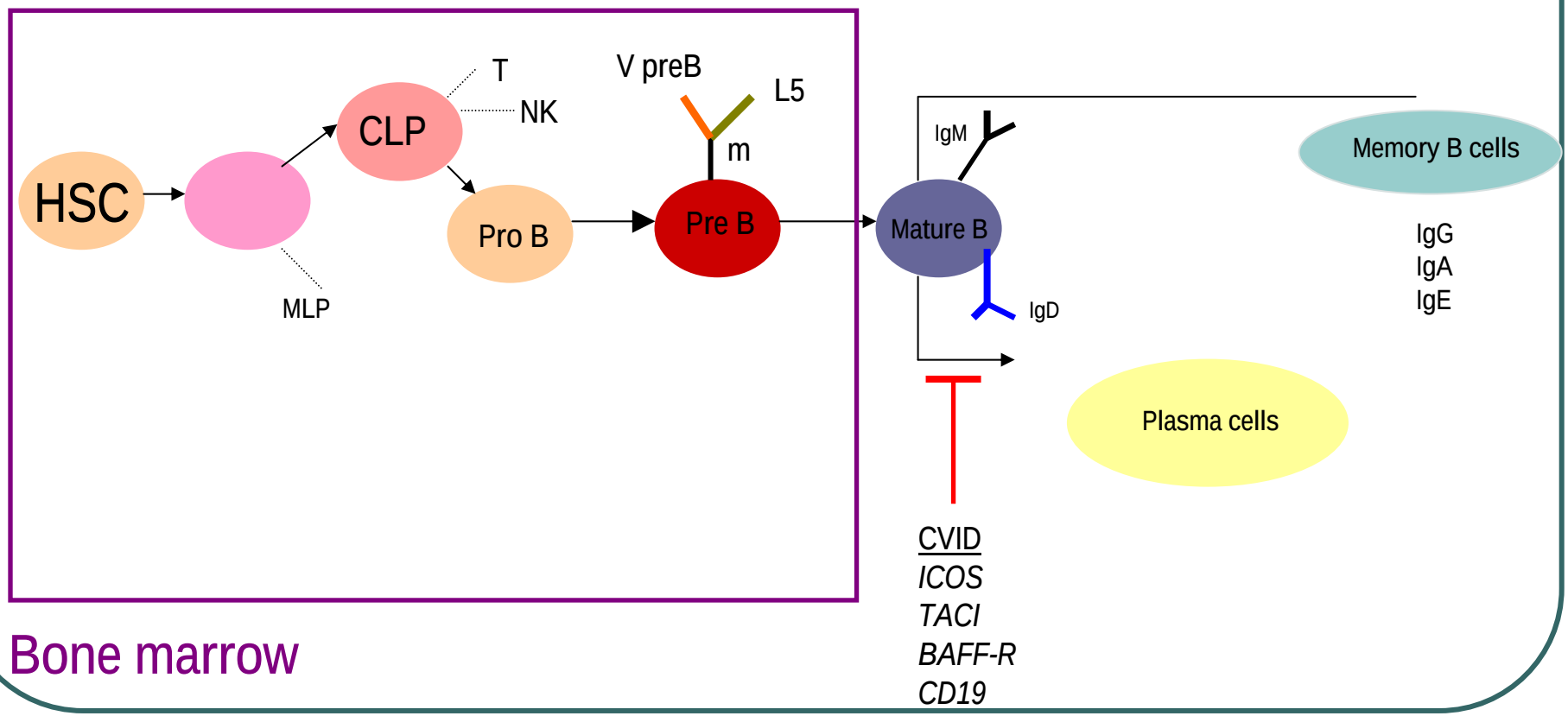
- absence of all immunoglobulins
- absence of B cells in periphery and bone marrow

VAŠEK Diagnosis and treatment

- mutation in a gene coding for Bruton tyrosinkinase (BTK)
- regular IVIg substitution
- currently without clinical symptoms
- prognosis relatively favourable

  (danger - echoviruses)

Hypogammaglobulinaemia



Fischer, Nature Immunology 2004

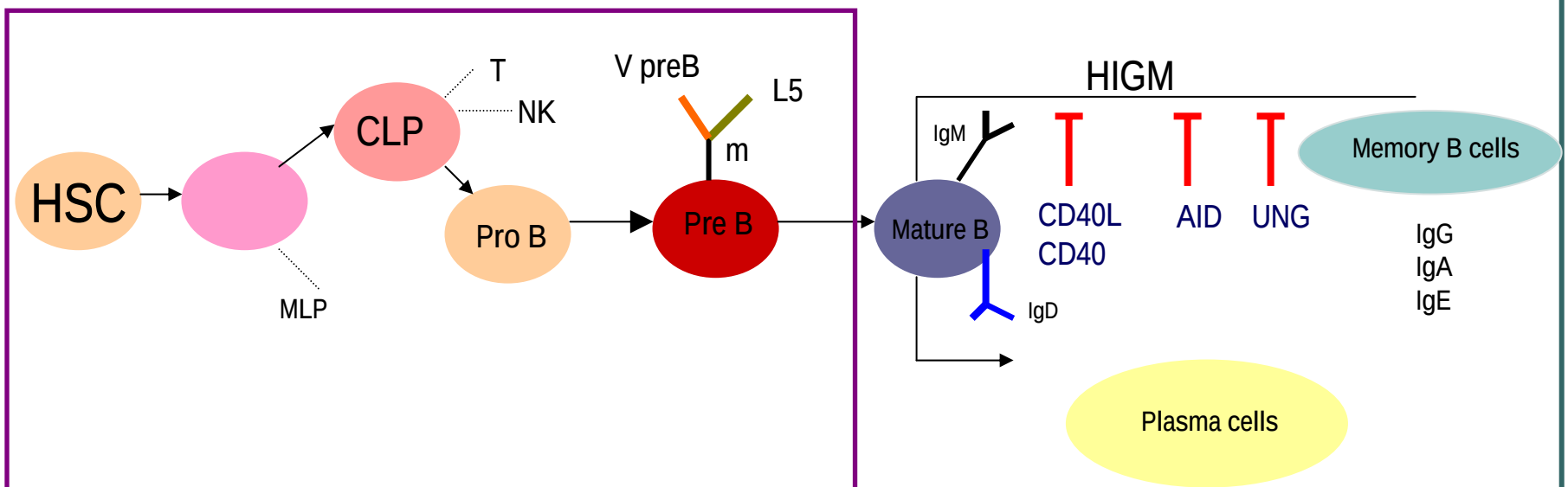
Common variable immunodeficiency (CVID)

- Male /female
- > 2 years
- Poor responses to vaccines
- Serum IgG and IgA are > 2 SD below mean for age
- Exclude other 2nd antibody deficiencies

Common variable immunodeficiency (CVID)

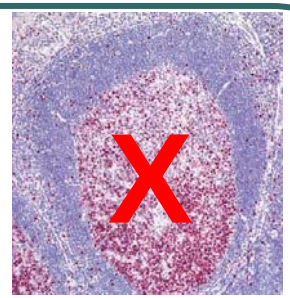
- highly heterogeneous
- manifestation later in life
- incidence 1 : 10.000 – 50.000
- **infections**, autoimmunity, granulomas
- ICOS (on T cells)
- TACI, BAFF-R, CD19 (on B cells)

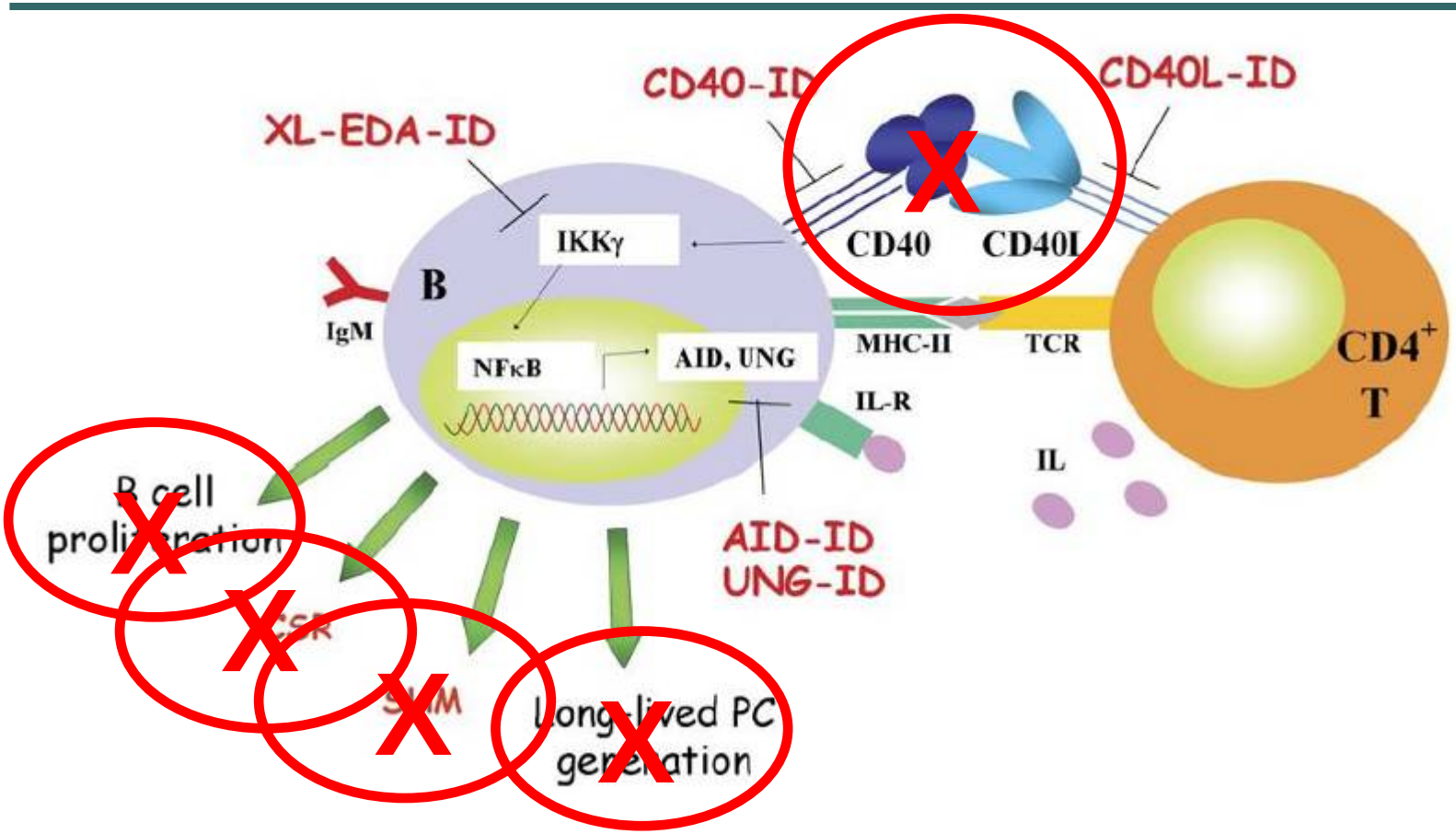
Defects of isotype switch



Fischer, Nature Immunology 2004

Defects in hyper IgM





Notarangelo, J Allergy Clin Immunol 2006, 117, 855-64

Hyper IgM syndrom (HIGM)

- bacterial and **atypical infection** (PCP, cryptosporidia)
- defect in communication (previously B cell defect expected)
- mainly boys (CD40L = X-linked)

ONDŘEJ, 8 month-old

ONDŘEJ Personal history

- family history unremarkable
- BCG vaccination
- in **3 months** **enlarged left axillar lymphnodes**
  ➡ suppuration ➡ drainage ➡ consolidation
- in **4 months** **cough**
- chronically **slurry yellow-green stool**
- since **2 months** **failure to thrive**

ONDŘEJ Disease detection

- in **8 months** oral thrush
- at GP: afebrile, **failure to thrive, thrush, tachypnoe**, clear breathing
- in hospital: at admission **sat. O_2 80%, leukocytosis**, trombocytosis, low ESR, low CRP

ONDŘEJ Immunological investigation

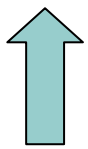
↓	**IgG 0,6**	g/l	[NR 3.6-7.7]
↓	**IgA < 0.06**	g/l	[NR 0.1-0.6]
↓	**IgE < 1**	IU/ml	[NR 0-30.0]
↑	**IgM 1,98**	g/l	[NR 0.3-1.4]

lymphocyte number

functional tests

- (proliferation)
- (phacytosis, NBT)

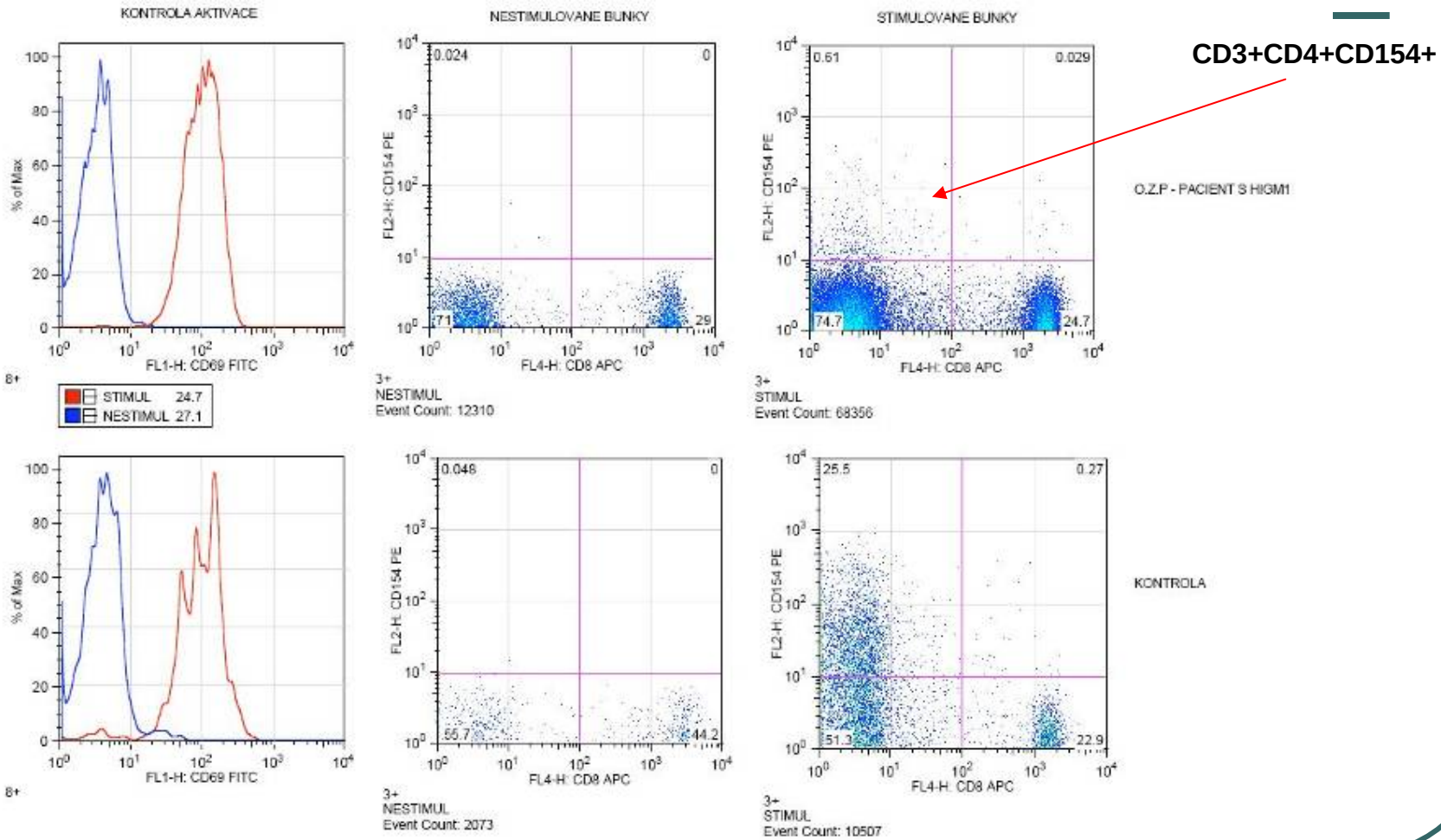
} normal

 **IgG, A, E + IgM** 

and atypical infection

Hyper IgM syndrome?

ONDŘEJ Before transplantation

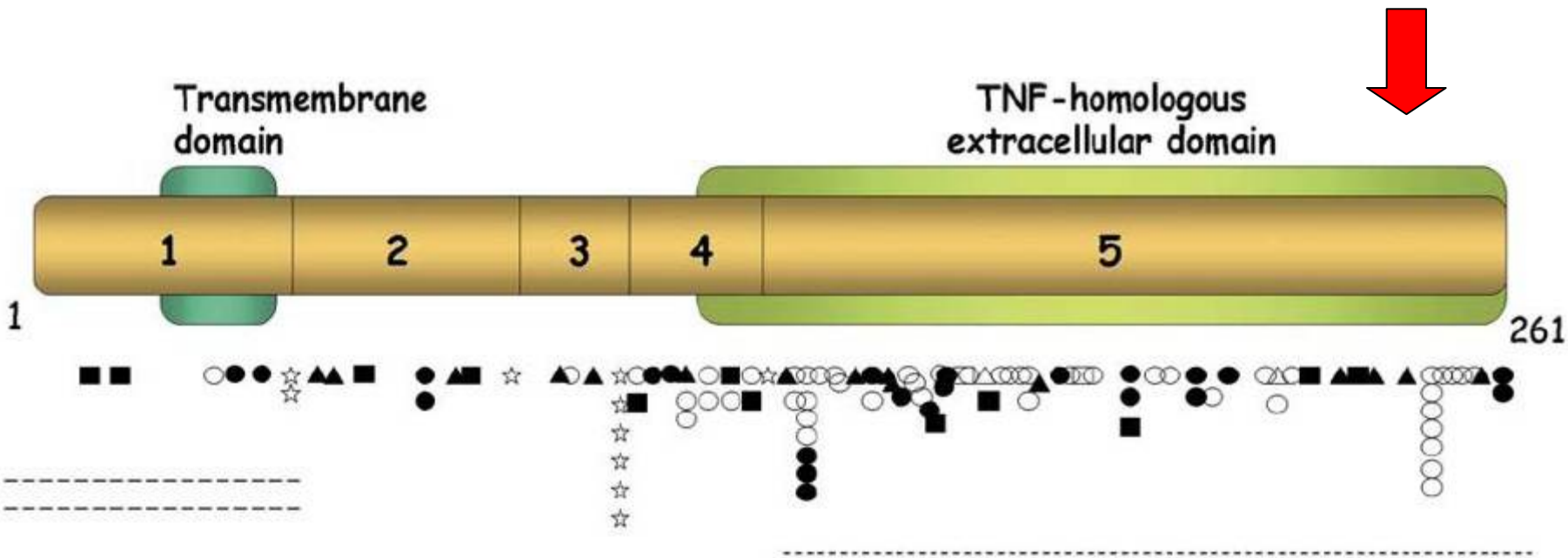


ONDŘEJ Molecular genetics

ONDŘEJ

mutation in exon 5, Cys800Thy
(dr. Genevieve de Saint Basil, Neckar, Pařris)

Xq26



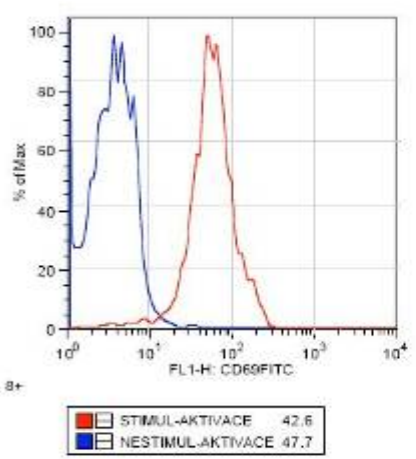
mother is CARRIER

Notarangelo, J Allergy Clin Immunol 2006, 117, 855-64

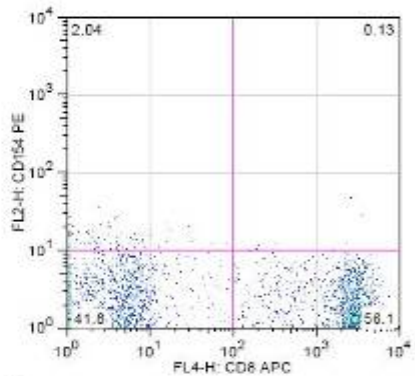
ONDŘEJ

After bone marrow transplantation

- KONTROLA AKTIVACE

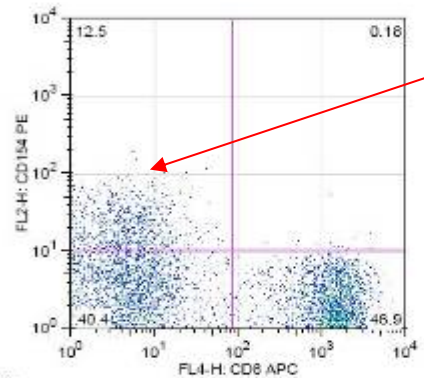


NESTIMULOVANE BUNKY



3+
NESTIMUL
Event Count: 3827

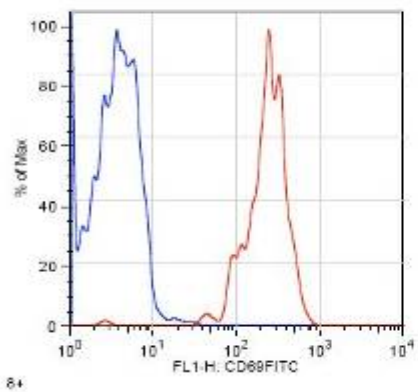
STIMULOVANE BUNKY

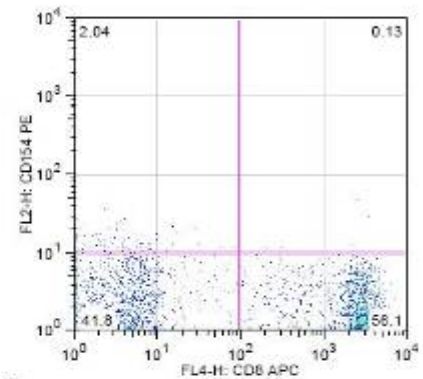


3+
STIMUL
Event Count: 6586

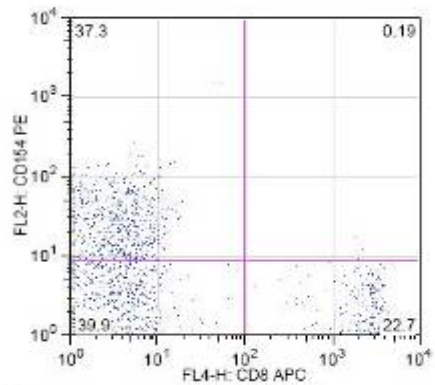
CD3+CD4+CD154+

O.Z.P. - PACIENT S HIGM1





3+
NESTIMUL
Event Count: 3827



3+
STIMUL
Event Count: 1598

KONTROLA

Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch
- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway
- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)
- Complement
  - particular components
  - regulatory factors
- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis
- Syndromes with compromised DNA repair

Cellular and combined PID

- severe combined (SCID)
  - cytokine signalization
  - T-cell receptor signalization
  - recombination of T-cell receptor genes
  - purine metabolism
  - expression of HLA molelules
- combined
  - intercelluar signalization
  - intracellular signalization
  - cellular motility
  - chemokine signalization
  - transcription factors
  - IFN gamma/IL-12 pathway

common gamma chain.
ZAP-70
RAG1/2, Artemis
ADA, PNP
HLA I, II

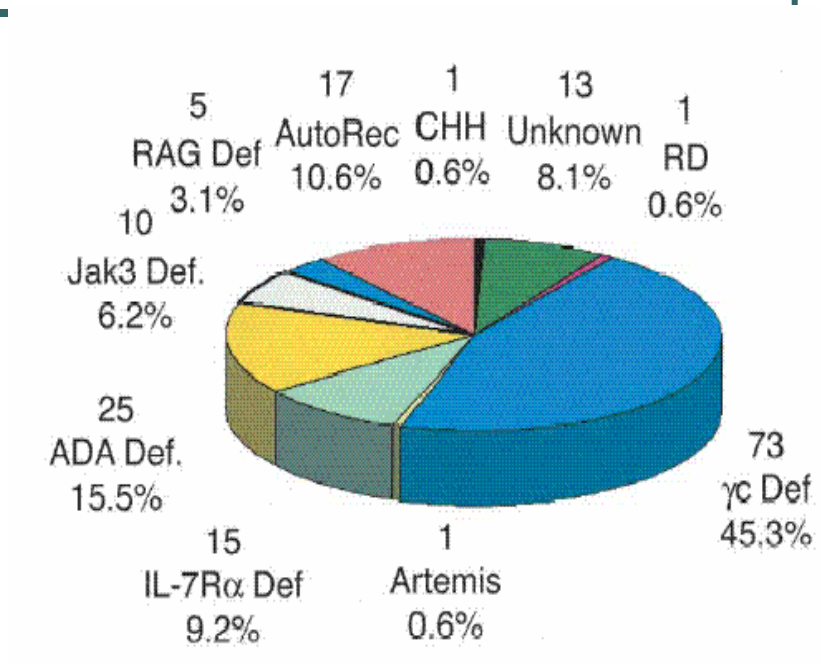
DiGeorge syndrome

Severe combined immunodeficiecy (SCID)

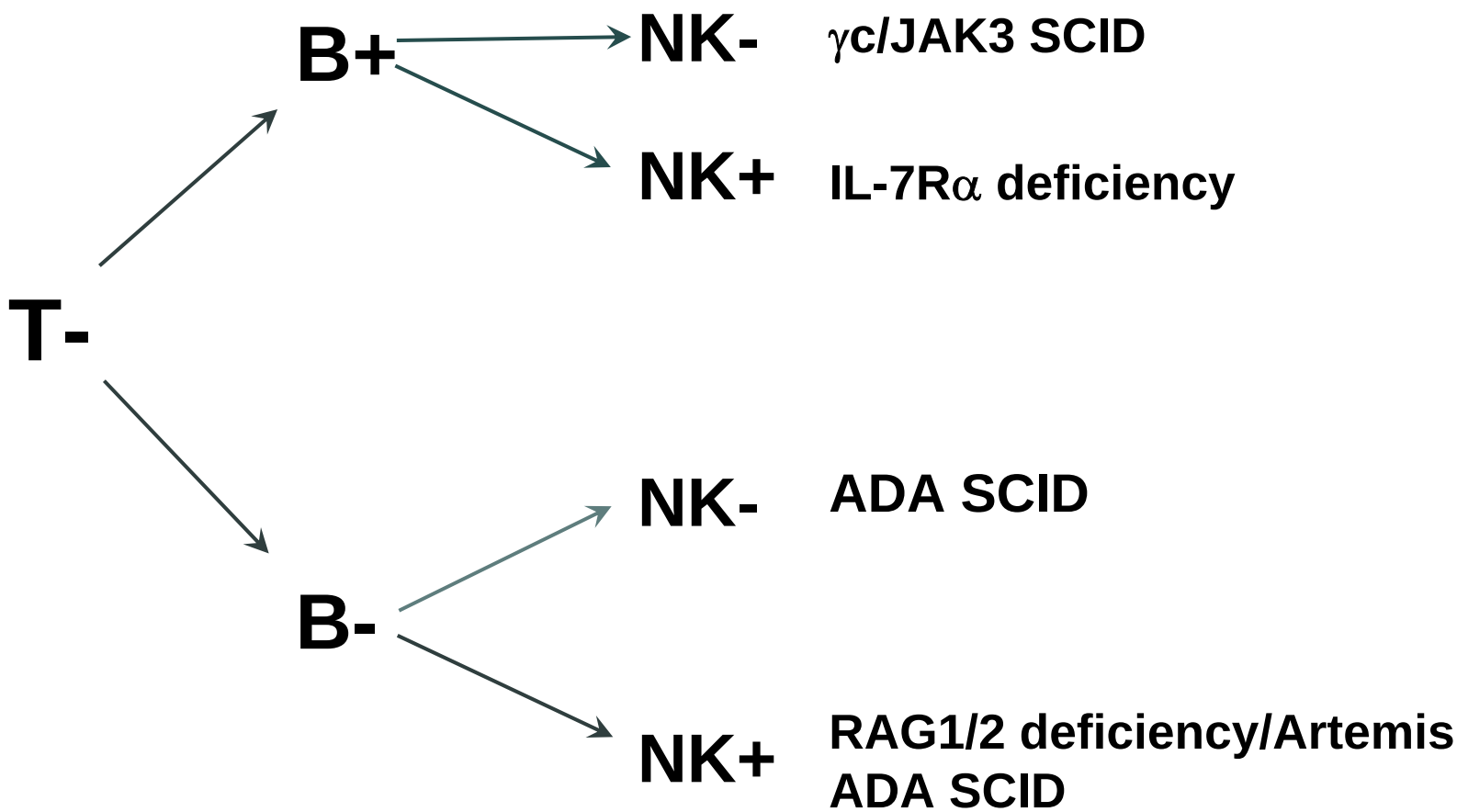
- clinical symptoms
  - early in life
  - chronic diarrhea, failure to thrive
  - graft versus host disease (on skin)
- complications after vaccination with live vaccines
- unusual infections, severe course
- family history

Laboratory findings

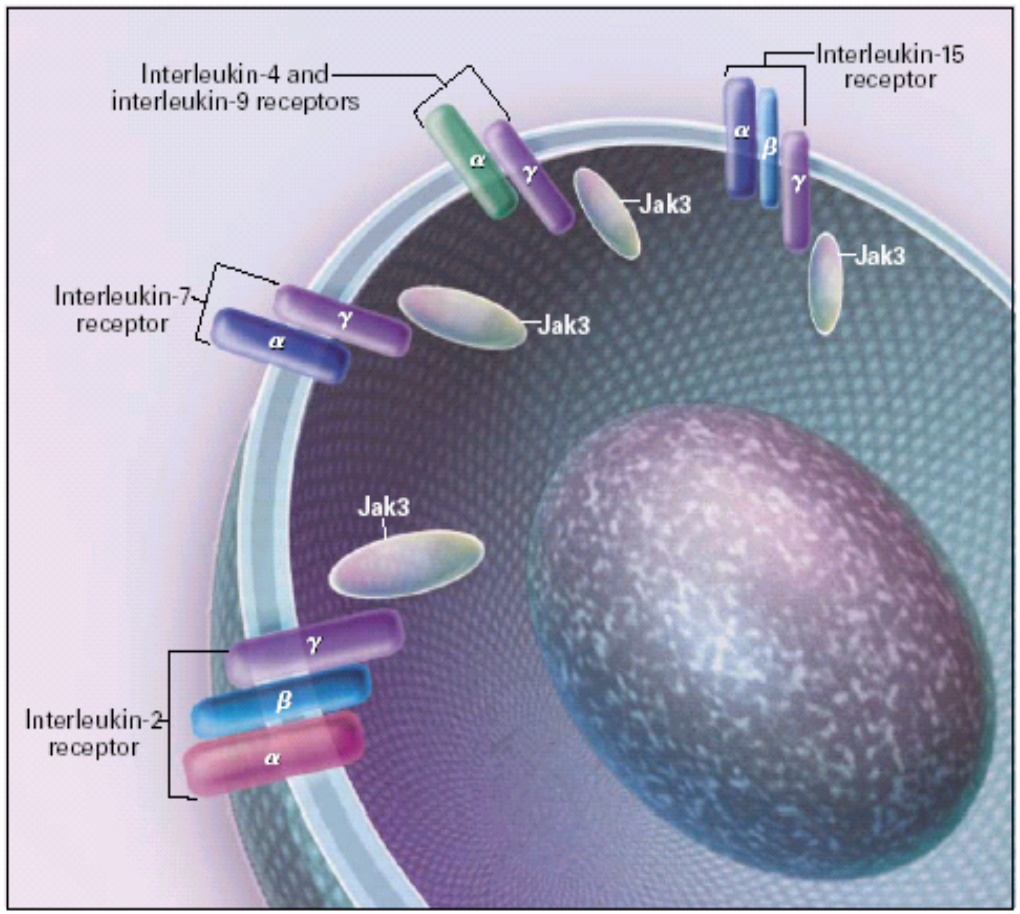
- lymphopenia
  - T-B-NK-
  - T+B-NK+
  - T-B+NK+
- defect in T-cell activation
  - e.g. n vitro PHA
- low serum immunoglobulins
  - beware – antibody transferred from mother



SCID


T-
B+
NK-
γc/JAK3 SCID
NK+
IL-7Rα deficiency
B-
NK-
ADA SCID
NK+
RAG1/2 deficiency/Artemis
ADA SCID

X-SCID


Interleukin-4 and
interleukin-9 receptors
α
γ
Jak3
Interleukin-15
receptor
α
β
γ
Jak3
Interleukin-7
receptor
γ
α
Jak3
Jak3
γ
β
α
Interleukin-2
receptor

MICHAL, 5 month-old boy

MICHAL Personal and family history

- in maternal family a few early deaths of boys
- properly vaccinated
- thrived well
- exanthema in 4 months
- admitted due to pneumonia

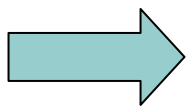
MICHAL Lab results

IgG 0

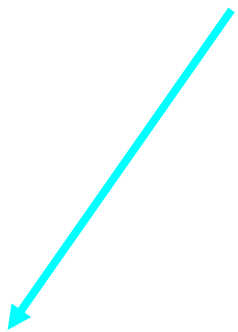
IgA 0

IgM 0.14

CD3+:	0.1%
CD3-16+/56+:	4.0%
CD19+:	96%



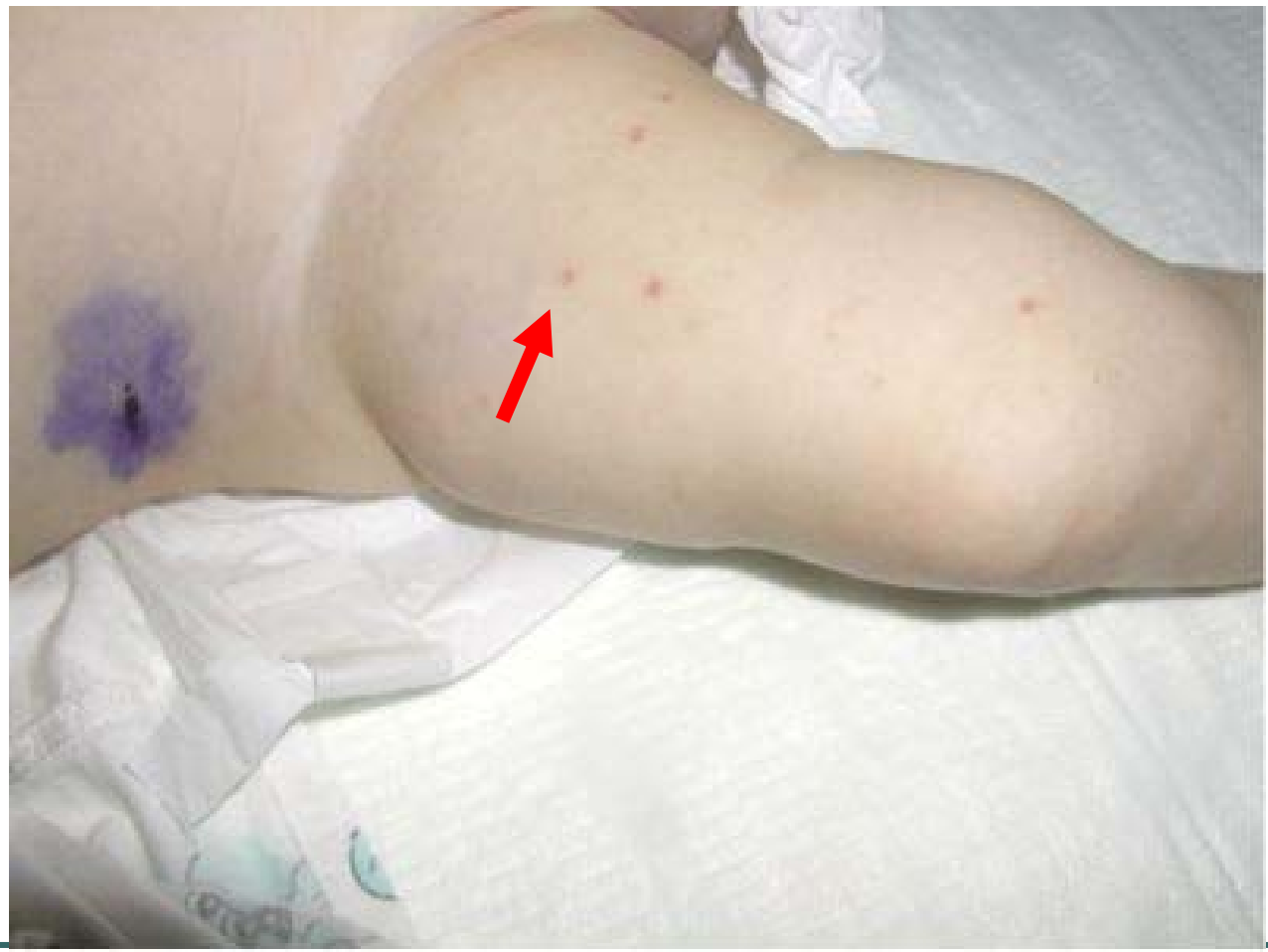
T-B+NK-



X-SCID

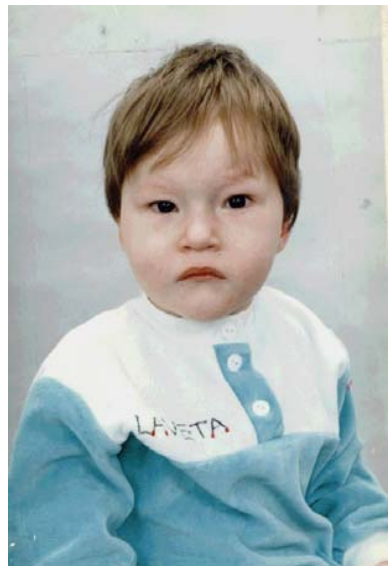
mutation in common gamma chain gene was found

MICHAL **Exanthema = BCGitis!**



DiGeorge syndrom - CATCH 22

cardiac defects
abnormal facies
thymic hypo/aplasia
cleft palate
hypocalcemia
deletion 22q11



Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch
- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway
- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)
- Complement
  - particular components
  - regulatory factors
- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis
- Syndromes with compromised DNA repair

Defect of Phagocytosis

- number of phagocytes
- adhesion
- function (intracellur killiing)

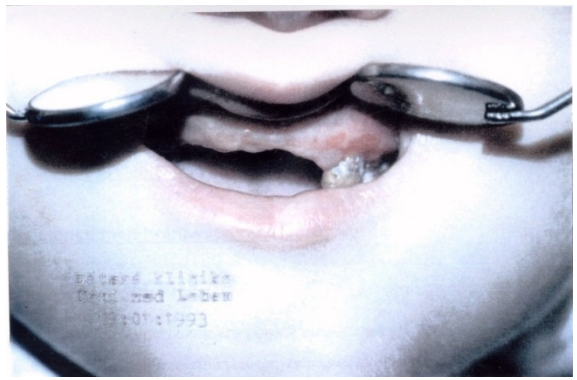
neutropenia (severe, cyclic)

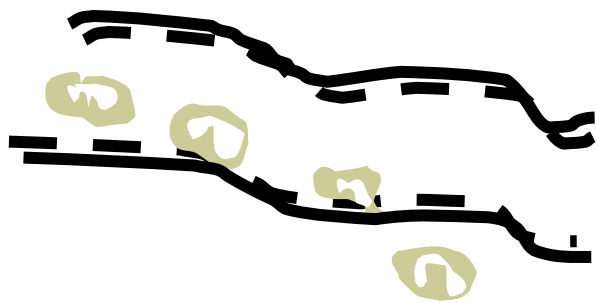
leukocyte adhesion defect (LAD)

chronic granulomatosis (CGD)

Leukocyte Adhesion Defect I

- subunit of superficial intergrin
- rolling is impaired
- persisiing leukocytosis
- delayed umbilical separation
- periodontitidis
- recurrent skin, respiratory and gut infections
- skin ulcers and necrosis
- first transplanted patient with PID in the Czech Republic (1994)

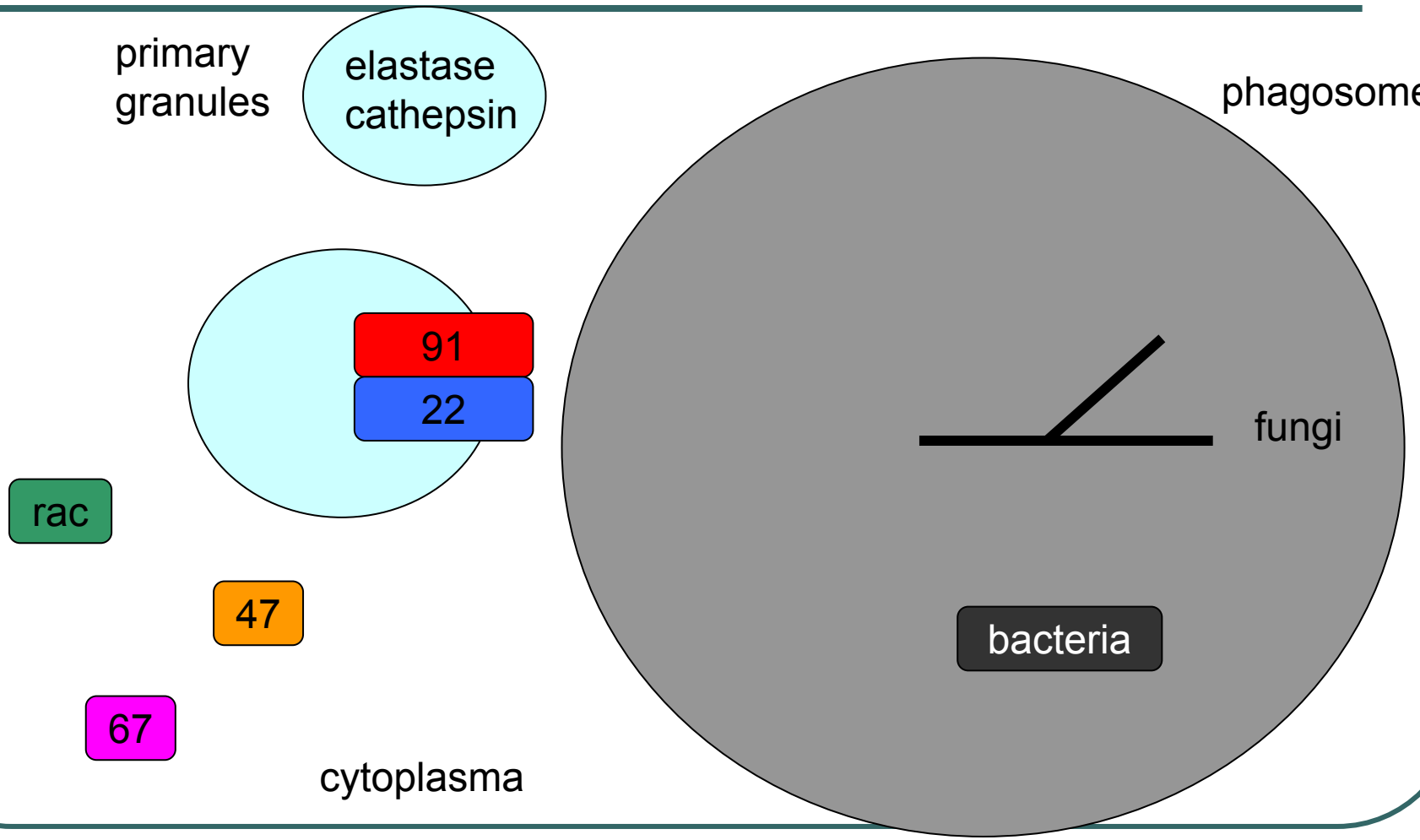




Defect of Phagocytosis
Chronic Granulomatous Disease

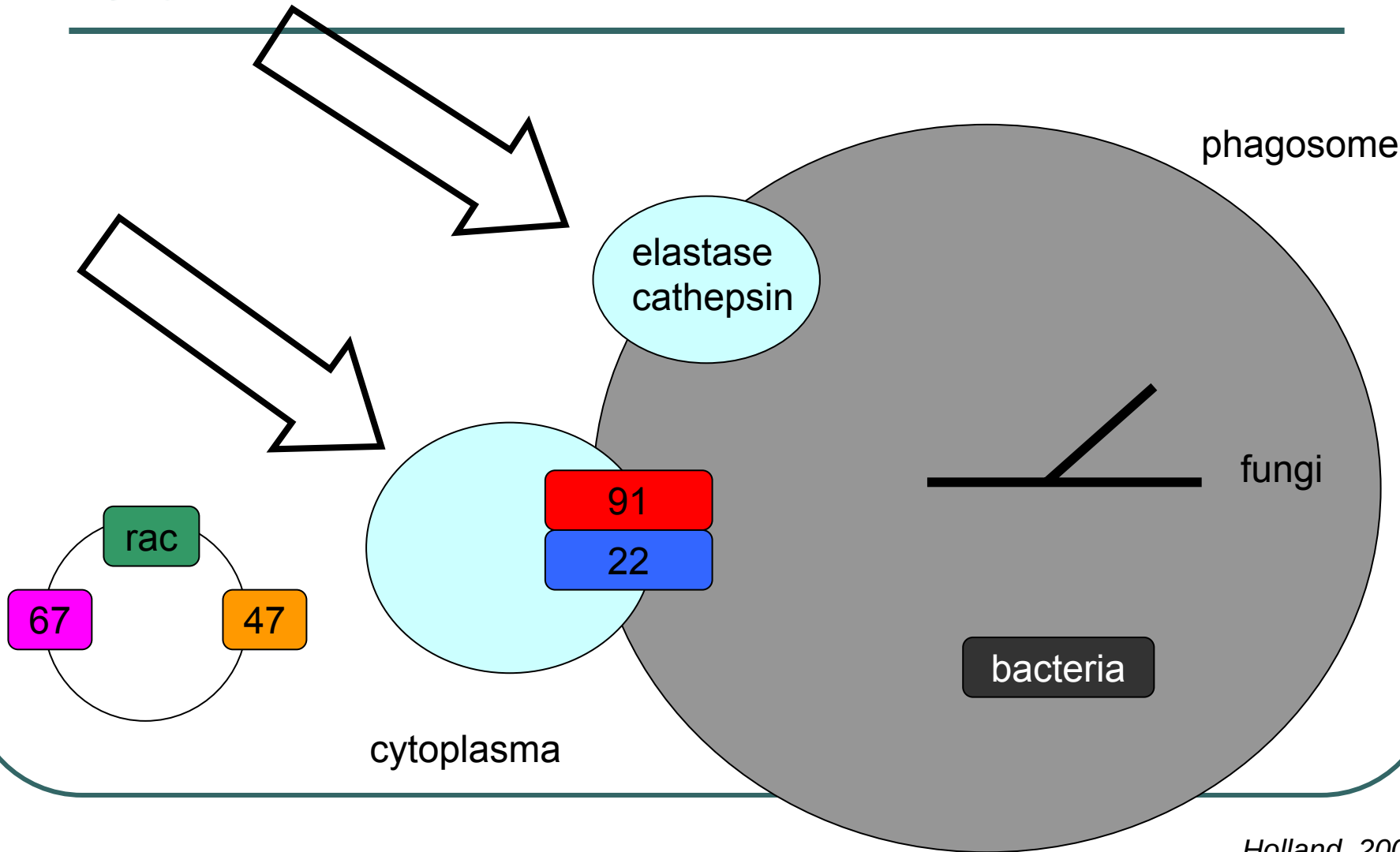
- inability of phagocytes to generete reactive oxygen radicals
- gene defect of one or more components of NADPH oxidase
- **X - linked**
  - defect in genefor gp91-phox -
  - membrane bound part of the molecule of cytochrom b558
- **autosomal recesive**
- defect in genes of membrane or
- cytoplasmatic subunits
- p47-phox, p67-phox, p22-phox

CGD



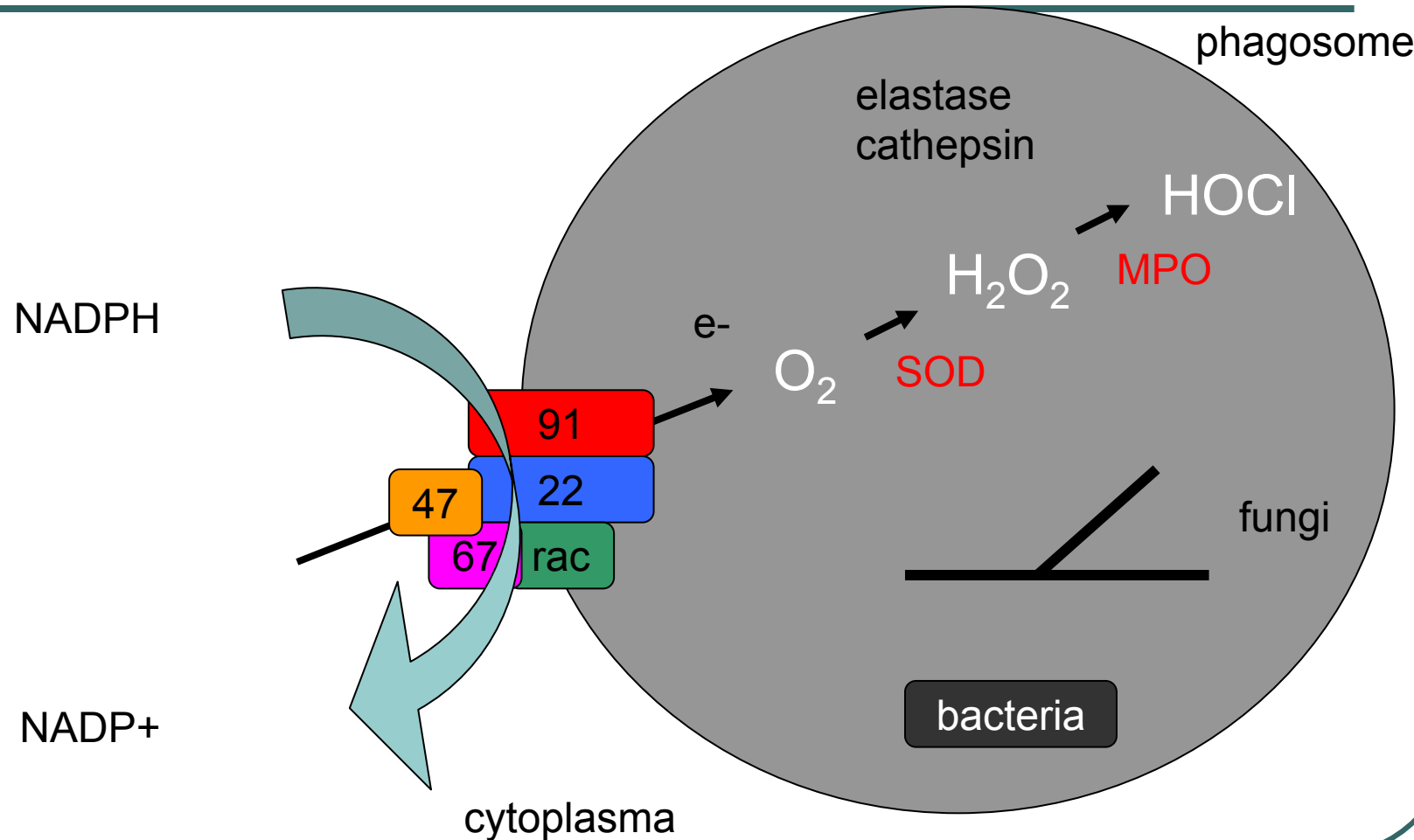
Holland, 2007

CGD



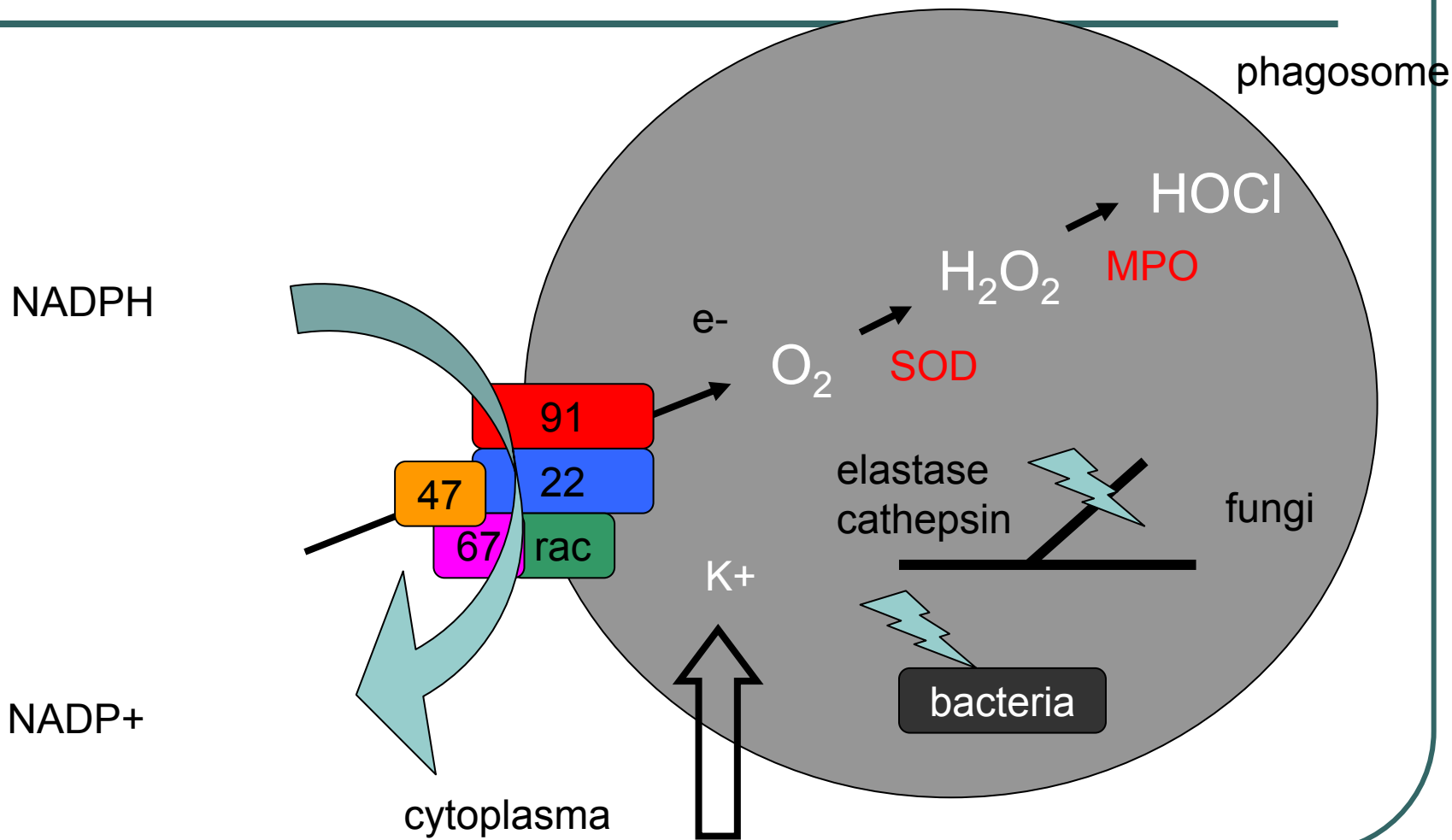
Holland, 2007

CGD



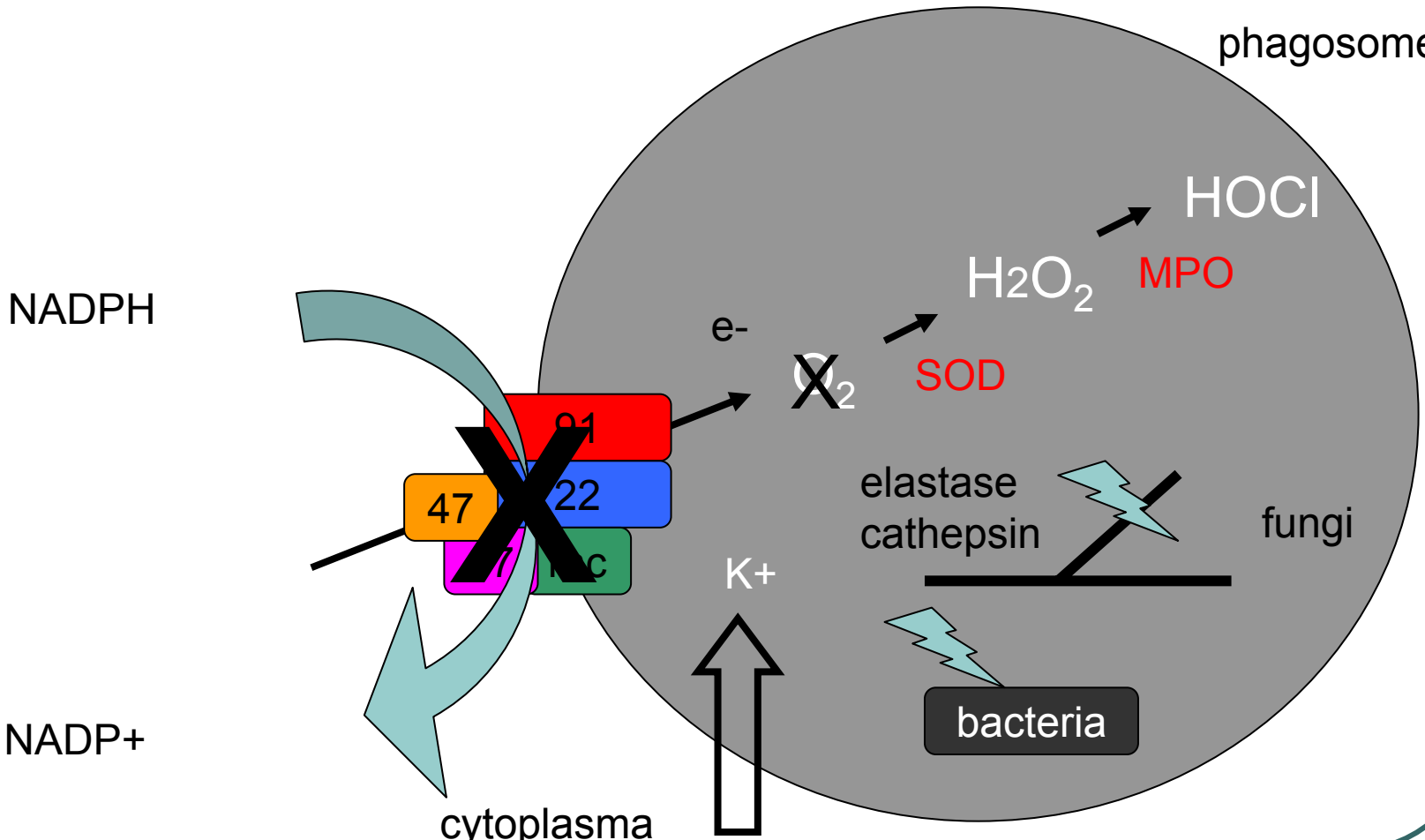
Holland, 2007

CGD



Holland, 2007

CGD



Holland, 2007

1 phenotype – 4 genotypes

X-CGD

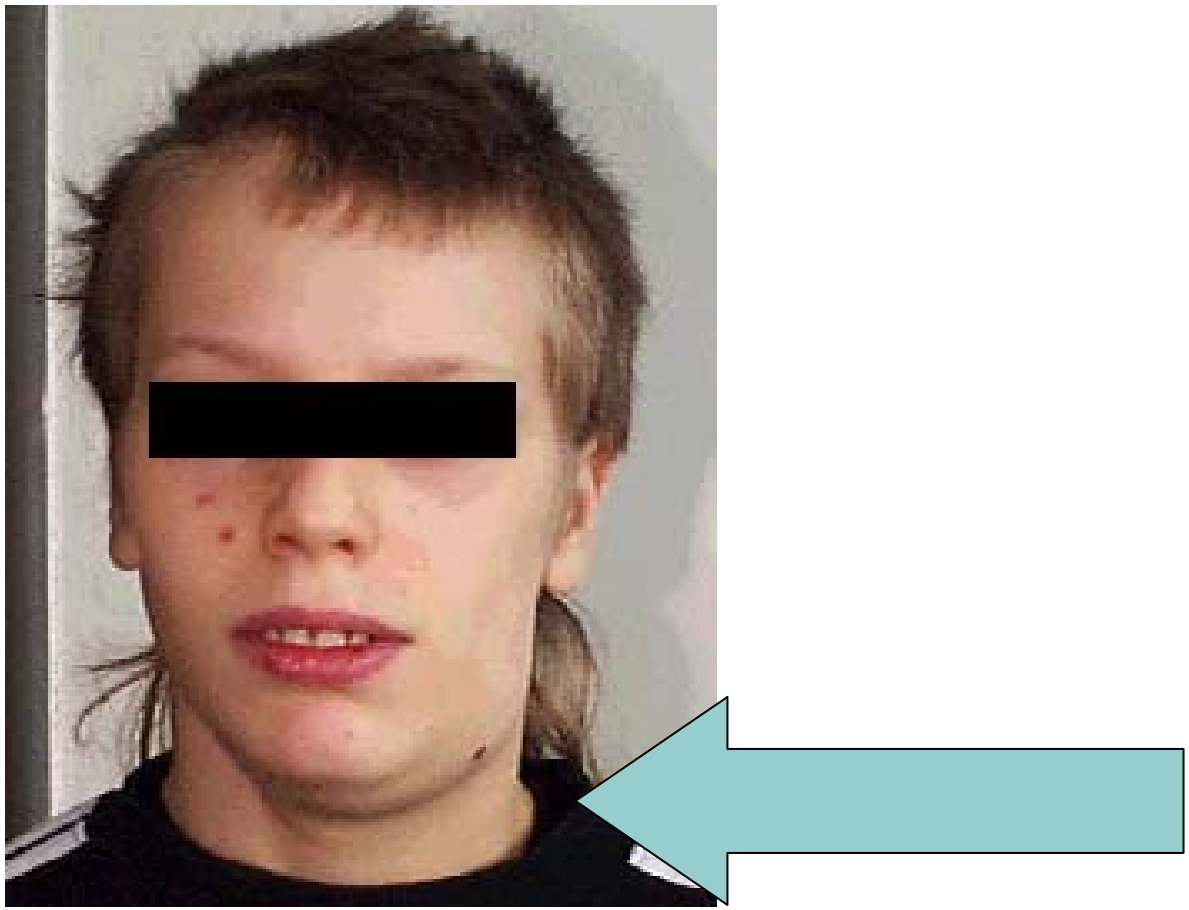
- CYBB, gp91^{phox} (X91^{0}, X91^{-}, X91^{+}) 65%

AR-CGD

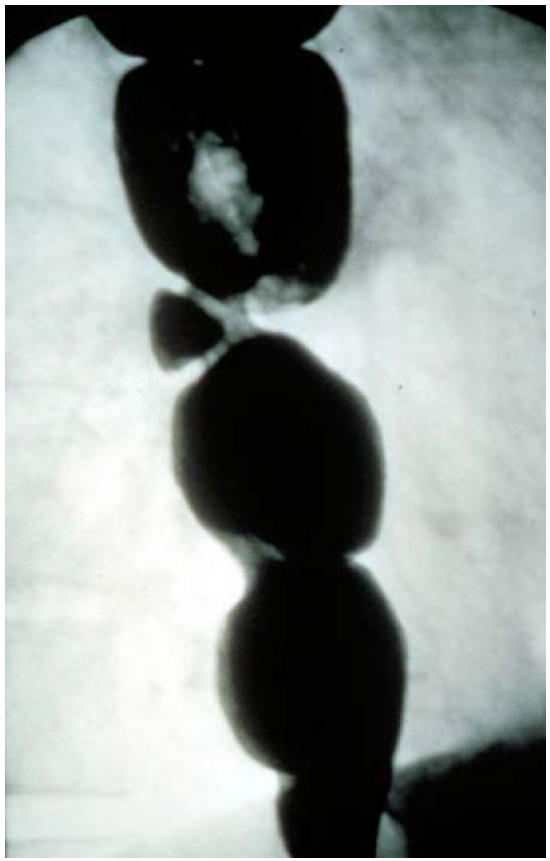
- CYBA, p22^{phox}, chr. 16 <5%
- NCF1, p47^{phox}, chr. 7 25%
- NCF2, p67^{phox}, chr. 1 <5%
  - milder course

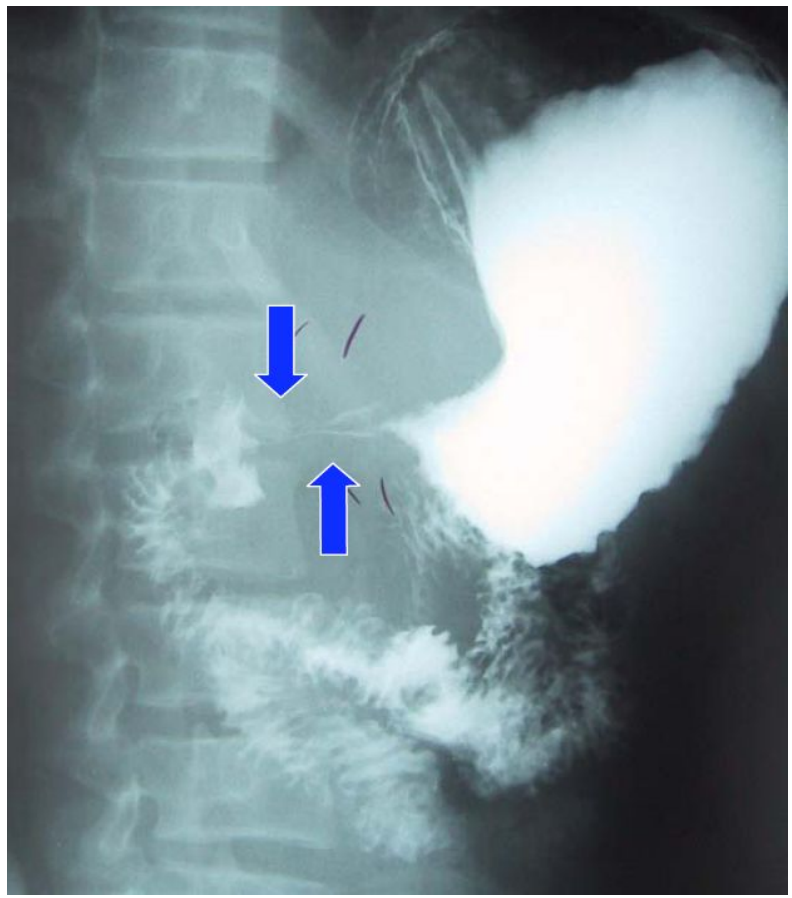
- incidence 1/100-200.000

Lymphadenitis



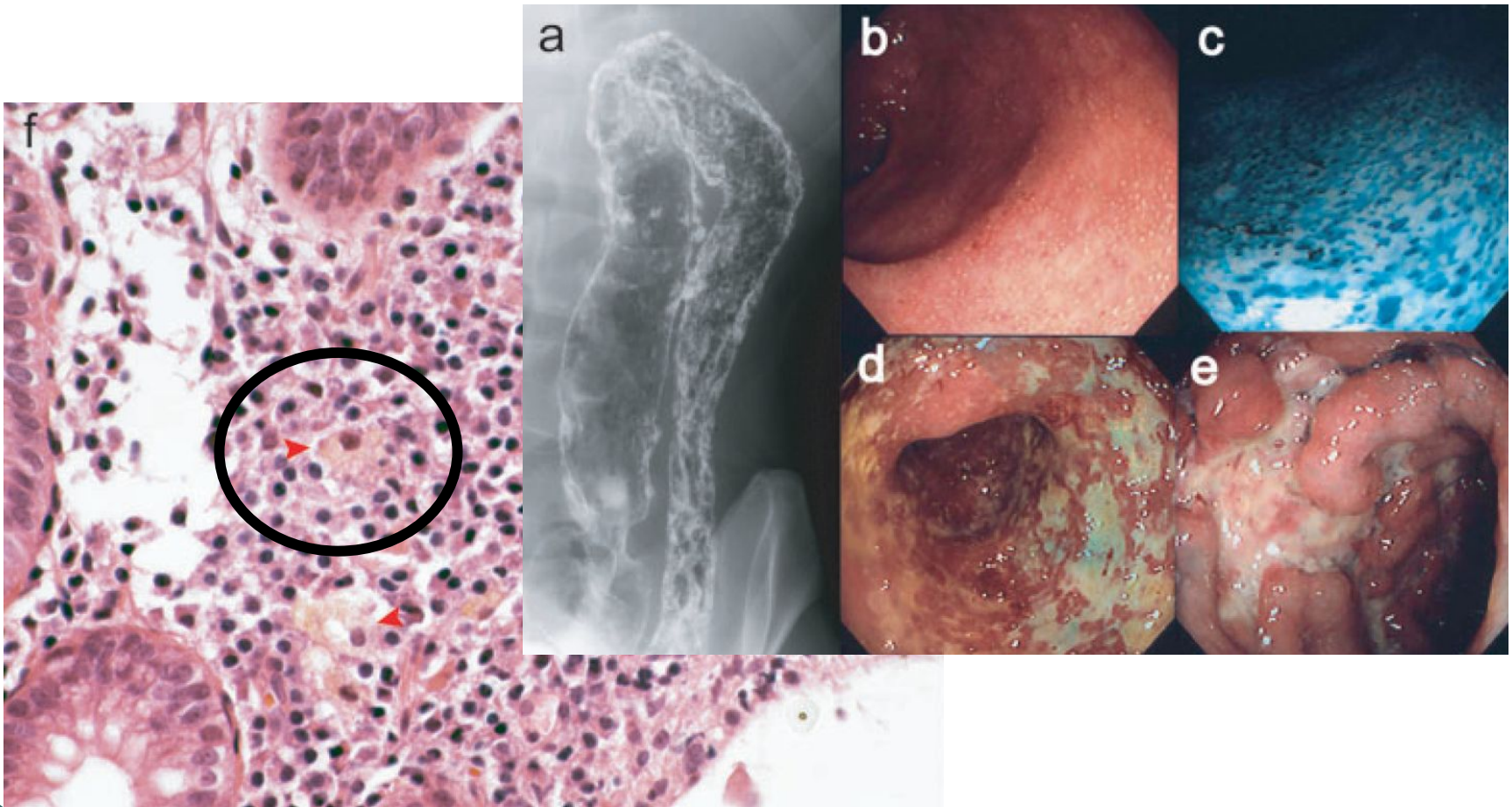
Granulomas leading to obstruction





Holland, 2007

Enterocolitis, Crohn-like



Arimura, 2006

Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch

- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway

- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)

- Complement
  - particular components
  - regulatory factors

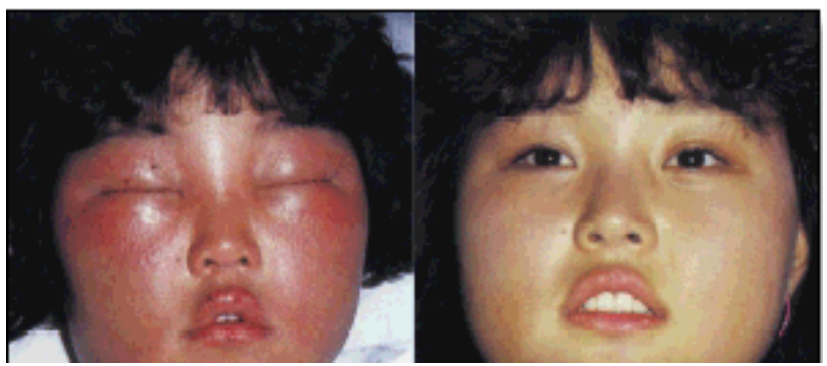
- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis

- Syndromes with compromised DNA repair

Complement deficiencies

- deficit of early components of complement cascade
- deficit of late components of complement cascade
- deficit in alternative pathway

- hereditary angioedema (deficit C1 inhibitor deficit)



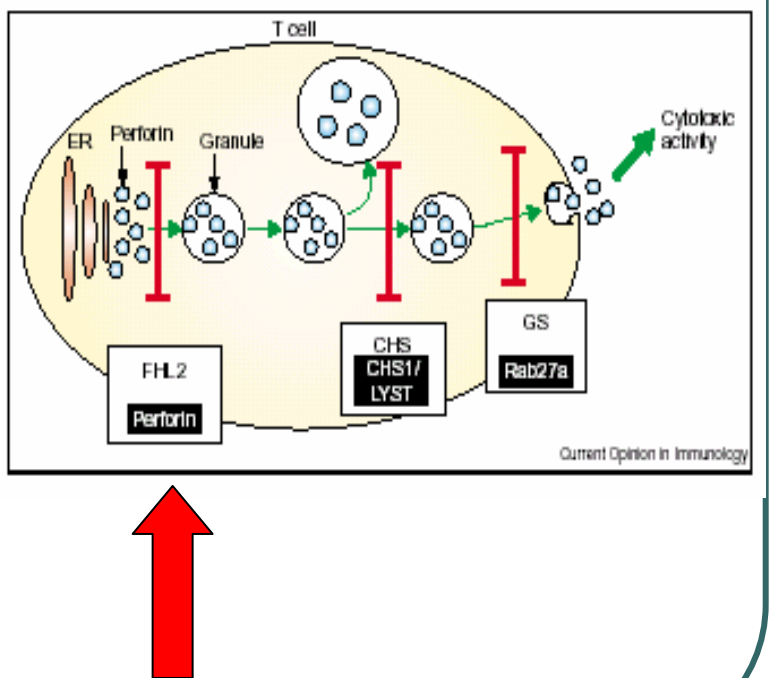
http://www.siamhealth.net/Health/Photo_teaching/urticaria.htm

Classification of primary immunodeficiencies

- Antibody
  - agammaglobulinaemia
  - hypogammaglobulineamia
  - deficit of specific antibodies
  - deficit of isotype switch
- Cellular, combined
  - severe combined (SCID)
    - cytokine signalization
    - T-cell receptor signalization
    - recombination of T-cell receptor genes
    - purine metabolism
    - expression of HLA molelules
  - combined
    - intercelluar signalization
    - intracellular signalization
    - cellular motility
    - chemokine signalization
    - transcription factors
    - IFN gamma/IL-12 pathway
- Phagocyte
  - number of phagocytes
  - adhesion
  - function (intracellur killiing)
- Complement
  - particular components
  - regulatory factors
- Malfunction of regulation
  - cytotoxicity
  - negative feedback
  - apoptosis
- Syndromes with compromised DNA repair

Familial hemophagocytic lymphohistiocytosis (FHL)

- first symptoms in previously healthy baby after infection (mainly EBV)
- low cytotoxicity
- highly activated CD8+ T-cells
- activation of macrophages leading to phagocytosis of BM cells



Prenatal Diagnostics

- history, affected family member
- PID with known molecular defect

- **XLA**
- **some forms of SCID**
- **chronic granulomatosis**

Investigation of patient with suspected PID

- history
- clinical examination
- laboratory investigation

Clinical presentation

- **Ask yourself**
  - **Severe**
  - **Persistent**
  - **Unusual**
  - **Recurrent**
- **Be guided by pathogens**
- **Beware of surprises !**

Lab tests to reveal PID

- B-cell function
  - Screening tests
    - Serum immunoglobulin levels
    - Serum specific antibody titers
  - Advanced tests
    - Antibody response to booster immunization
    - Flow cytometry to enumerate B cells
    - In vitro immunoglobulin production in response to mitogen
    - In vitro immunoglobulin production in response to anti-CD40 and cytokines
    - Antibody response to immunization with ϕ X174
- Cellular immune function
  - Screening tests
    - Flow cytometry to enumerate T cells and natural killer cells
    - Cutaneous delayed hypersensitivity
  - Advanced tests
    - Enzyme assays (ADA, PNP)
    - FISH for 22q11 and 10p11 deletion
    - In vitro proliferative response to mitogens and antigens
    - Natural killer cell cytotoxicity
    - Cytokine production in response to mitogen or antigen stimulation
    - Expression of surface markers after mitogen stimulation
- Phagocytic cell function
  - Screening tests
    - Blood cell count with differential
    - Neutrophil staining, morphology
  - Advanced tests
    - Oxidase function (dihydrorhodamine, nitroblue tetrazolium, chemiluminescence)
    - Flow cytometry for adhesion molecules
    - Chemotaxis
    - Phagocytosis
    - Enzyme assays (myeloperoxidase, G6PDH)
    - WBC turnover
    - Bacterial or fungal killing
    - Bone marrow biopsy
- Complement function
  - Screening tests
    - CH_{50} (total hemolytic complement activity)
    - AH_{50} (alternative pathway hemolytic activity)
  - Advanced tests
    - Level or function of individual complement components
    - Chemotactic activity of complement split products
- General
  - Advanced tests
    - Molecular methods including Southern, Northern, and Western blots, PCR/SSCP, DNA fingerprinting, and nucleotide sequencing

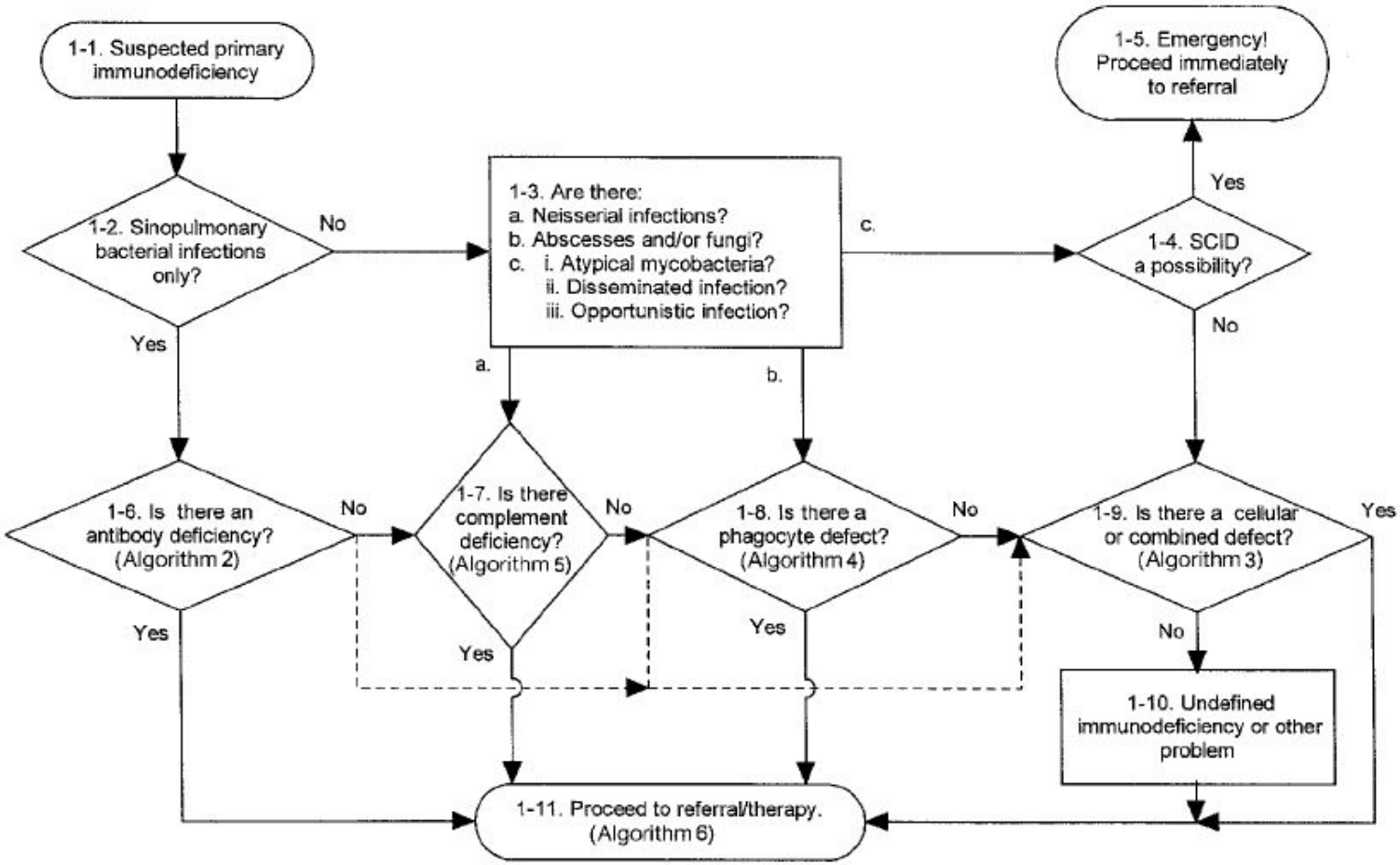
Differential diagnosis of PID

	HUMORAL	CELLULAR COMBINED	PHAGOCYTE	COMPLEMENT
Frequency	70%	20%	9%	1%
Age	<6 m	0 ... < 2 y	0 ... < 2 y	all
Symptoms	respiratory inf. otitides pneumonias arthritides	severe respiratory inf. pneumonias dermatitis diarrhoea	omphalitis adenitis pyodermia otitides	autoimmunity SLE pyogenic inf. oedema
Infections	extracellular b. echoviruses	viruses fungi mycobacteria	staphylococci fungi enterobacteria	neisseria

Differential diagnosis of PID

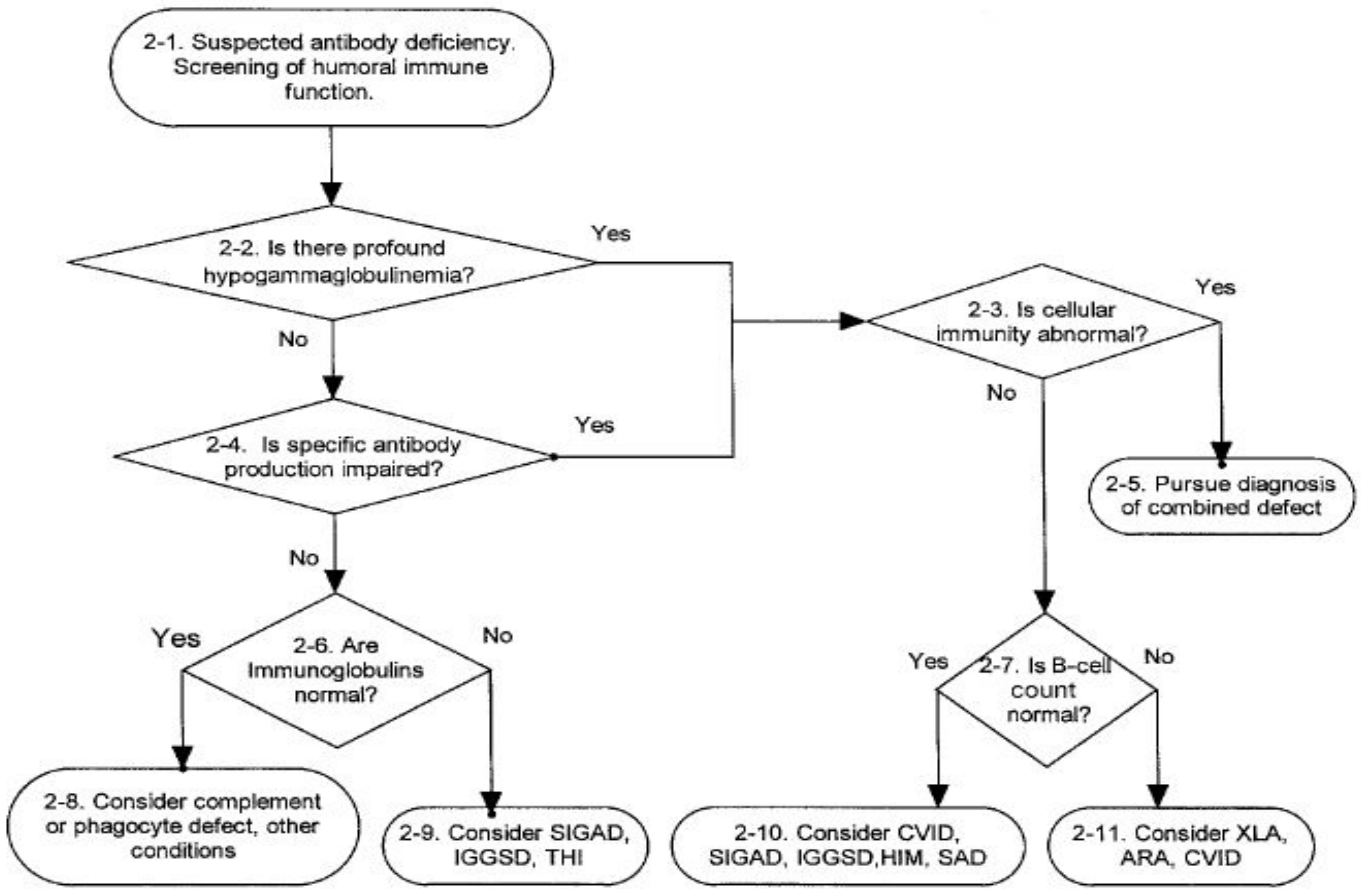
	HUMORAL	CELLULAR COMBINED	PHAGOCYTE	COMPLEMENT
Complications	cardiovascular echoviral inf.	infections tumors autoimmunity	infections	various
Survival	adulthood	early childhood	individual	individual
Diseases	XLA CVID sIGAD	SCID	CGD LAD	HAE

General approach in diagnosis of PID



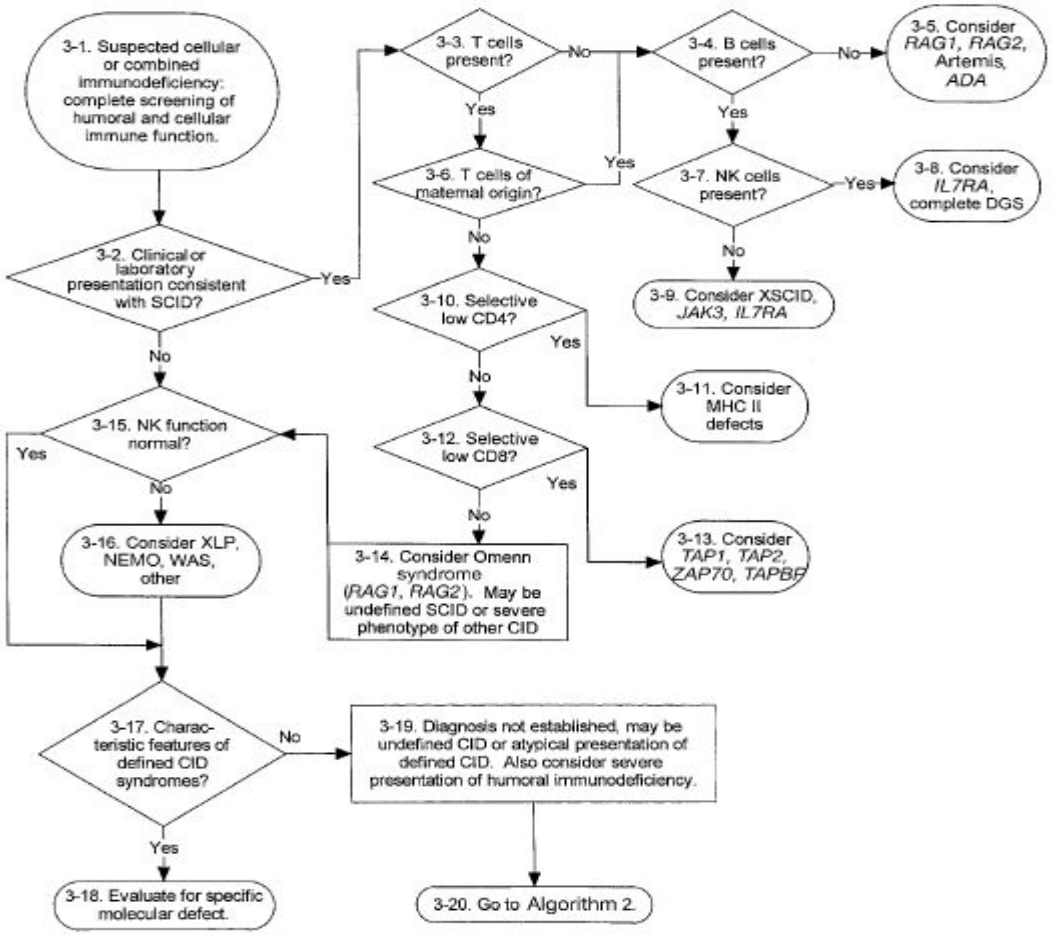
Bonilla, 2005

Diagnosis of humoral PID



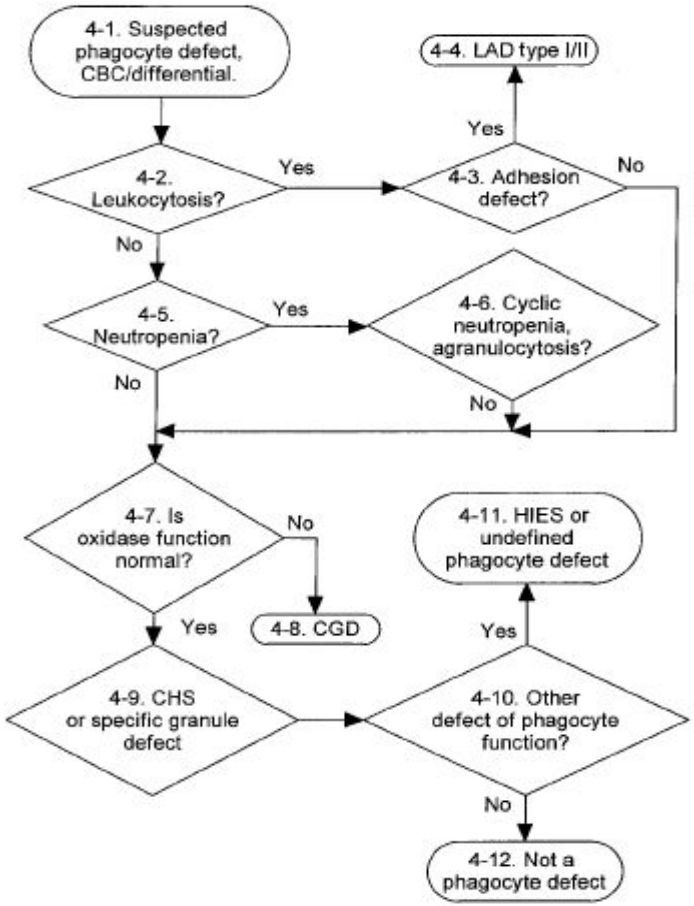
Bonilla, 2005

Diagnosis of cellular PID



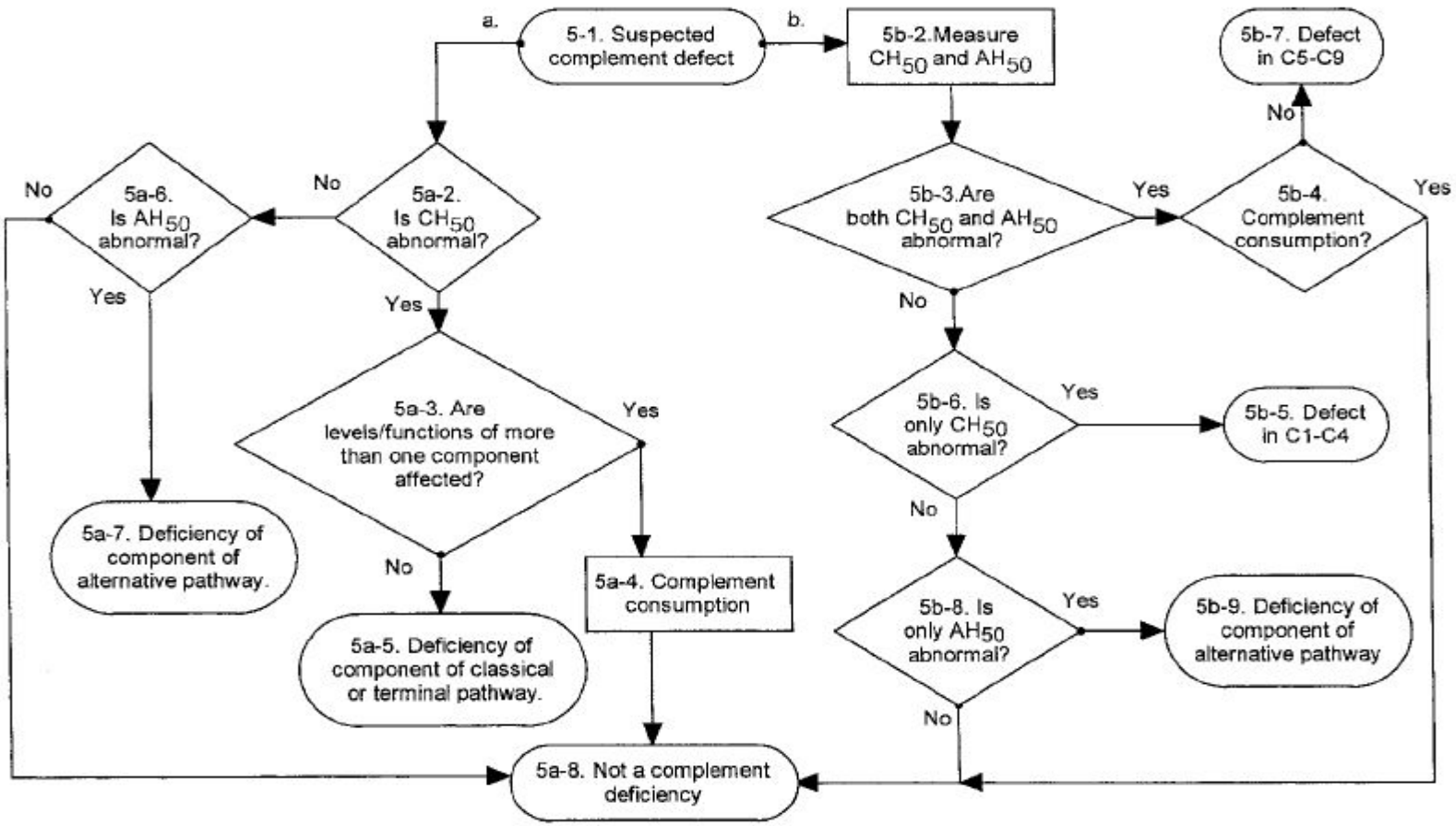
Bonilla, 2005

Diagnosis of phagocyte PID



Bonilla, 2005

Diagnosis of complement PID



Bonilla, 2005

Therapy of PID

- IVIG
- antibiotic and antimycotic therapy
  - chronic granulomatosis
  - LAD
- bone marrow transplantation
  - SCID
  - LAD
  - Wiskott-Aldrich syndrome
- Interferon gamma
  - chronic granulomatosis

G-CSF a GM-CSF

neutropenia

gene therapy

ADA deficiency

X -linked SCID

(LAD, chronic granulomatosis.)

other forms of therapy

Resources

- **Slatter** et al., Clin Exp Immunol, 2008, pp. 389-96
- **deVries** et al., Clin Exp Immnol, 2006, pp. 204-14
- **Notarangelo** et al., J Aller Clin Immunol, 2005, pp 883-96
- **Bonilla** et al., Practice parameters for the diagnosis and management of primary immunodeficiecy, Annals of Asthma, Allergy and Immunology, 2005, S1-S65
- **Ochs** et al., Primary immunodeficiency Diseases, 2nd edition, Oxford, 2006

ales.janda at lfmotol.cuni.cz