Genetics of Primary Immunodeficiencies

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Learning Objectives

• Define and classify primary immunodeficiency diseases → changing definition

• Review the role of clinical laboratory in the diagnosis of primary immunodeficiency diseases

• Demonstrate the utility of molecular diagnosis in primary immunodeficiency diseases → changing methodology

• Review examples of genetic susceptibility to bacterial, fungal and viral infections:
  - Mendelian suscept. to chronic mucocutaneous candidiasis
  - Mendelian susceptibility to mycobacterial infections
  - Genetic susceptibility to herpes simplex encephalitis
Definitions

• Primary immunodeficiency (PID): genetic

• Secondary immunodeficiency: infection, malignancy, iatrogenic
Primary antibody deficiencies are the most common PIDs

European ESID patient registry 2010

Antibody deficiencies:
Diagnosis ≥16yo = 86%
Diagnosis ≤15yo = 46%


http://www.esid.org/statistics
• Prevalence: 86.2/100,000
• Incidence: 10.3/100,000
(Joshi et al. 2009; Boyle and Buckley 2007)
PID

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- Incidence: 10.3/100,000
  (Joshi et al. 2009; Boyle and Buckley 2007)

Leukemias
- Prevalence: 81.6/100,000
- Incidence: 12.5/100,000
Identification of patients with PID

• Diagnosis of PIDs requires integration of data from clinical findings with laboratory immunological analyses and genetic testing

→ Infections
  - recurrent
  - life-threatening
  - unusual

→ Autoimmune diseases

→ Malignancies
Diagnosis of PIDs

Clinical and family history-based diagnostic approach

Diagnostic immunology lab supports, refine phenotype

Molecular lab: diagnosis, genotype-phenotype, etc
Recur. respiratory infections w/ diarrhea

B cell and Ig. evaluation

- Immunoglobulins
- Flow cytometry

Severe viral/fungal infections

T cell evaluation

- Lymphocyte prolif.
- Flow cytometry

Abscesses, delayed separation of umbilical cord

Granulocyte evaluation

- CBC, DHR, MPO
- Flow cytometry
Recur. respiratory infections w/ diarrhea
Severe viral/fungal infections
Abscesses, delayed separation of umbilical cord

B cell and Ig. evaluation
T cell evaluation
Granulocyte evaluation

- Immunoglobulins
- Flow cytometry
- Lymphocyte prolif.
- Flow cytometry
- CBC, DHR, MPO
- Flow cytometry

When to do mutation analysis?
Mutation analysis in PIDs

- Definitive diagnosis (atypical presentations)
- Presymptomatic identification
- Carrier identification, genetic counseling, prenatal diagnosis
- Prognosis (strong genotype-phenotype correlation)
- Technical considerations (DNA stability vs. live cells)
Primary immunodeficiencies

PID are like other genetic diseases, but

- secondary causes of ID are more common than primary (esp. in adults)

- manifestations (infections) can be the same in both primary and secondary ID as well in “healthy” individuals
Primary immunodeficiencies

- PID: changing definition
  from very severe Mendelian traits to varying degree of susceptibility to infections, including susceptibility to single infectious agents
  → not all-or-nothing (normal vs. immune deficient)
- ~150 recognized PIDS
- >200 genes (increasing fast)
  - candidate genes
  - mapping studies
  - novel technologies (genome/exome sequencing)
- Pathways not genes
Candida albicans is present in GI flora and reproductive mucosa of healthy subjects.

Immunocompromised patients C albicans can cause systemic or mucosal disease.

Systemic candidiasis is an acute, disseminated, and invasive form mainly in patients with inherited or acquired neutrophil and T cell disorders.

- Candidemia is one of the most prevalent bloodstream infections in hospital settings and is associated with significant morbidity and mortality.

CMC is candidiasis localized to the skin, nails, and mucous membranes (no predisposition to invasive disease, such as sepsis or pneumonia).
Predisposition to systemic candidiasis

- invasive surgery
- total parenteral nutrition
- diabetes mellitus
- intensive care conditions
- central lines
- neoplasia
- long-term use of broad-spectrum antibiotics or immunosuppressive agents
- HIV
- any global T-cell deficiency or neutrophil disorders
Immunity to candida

- Impaired T cell immunity: HIV/AIDS, SCID, DiGeorge syn., DOCK8 deficiency, etc.

- Congenital neutropenias or secondary neutropenia (e.g. after chemotherapy)

- Recognition:
  - mannans (outer portion of the cell wall)
    → TLR4, Mannose Receptor, DC-SIGN, and Dectin2
  
  - β-glucans (inner portion of the fungal cell wall)
    → Dectin1, and MINCLE
Genetics of CMC

- Dectin-1 deficiency (AR)
- CARD-9 deficiency (AR)
- AD Hyper IgE syndrome or Job syndrome: STAT3 deficiency
- AR Hyper IgE syndrome: DOCK8 deficiency
- STAT1 mutations (AD)
- IL-17F deficiency (AR)
- IL-17RA deficiency (AR)
- APECED (or APS type I): AIRE deficiency (AR)

Molecular testing → diagnosis, prognosis, inheritance

Immune response: receptors → signaling → effector functions
Fungal PAMPs

O-linked mannans, Zymosan

Toll-Like Receptors

TLR2  TLR4

DC-SIGN  MINCLE

Mannose R

β-glucan

Dectin1*

Dectin2

N-linked mannans

C-type lectin receptors

MyD88

NF-κB activation

CARD9*

Signaling complex

Signaling complex

IL1β

Pro-IL1β

Th17 priming cytokines

Co-stimulatory molecules
Fungal PAMPs

- **O-linked mannans, Zymosan**
- **β-glucan**
- **N-linked mannans**
  - Dectin1*
  - Dectin2
- **C-type lectin receptors**

Toll-Like Receptors

- **TLR2**
- **TLR4**

**Naïve T cells**

**MyD88**

Signaling complex

**CARD9***

Signaling complex

**NF-κB**

**IL1β**, **IL6**, **IL23**, **TGFβ**

**Pro-IL1β**

**Th17 priming cytokines**

**Co-stimulatory molecules**
Fungal PAMPs

O-linked mannans, Zymosan

Toll-Like Receptors

TLR2, TLR4

DC-SIGN, MINCLE, Mannose R

β-glucan

Dectin1*

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N-linked mannans

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Signaling complex

IL1β, Pro-IL1β

Th17 priming cytokines

Co-stimulatory molecules

STAT1, STAT3*

Naïve T cells

IL1β, IL6, IL23, TGFβ

IL17A/F

Th17 cells
**Fungal PAMPs**

- **O-linked mannans, Zymosan**
- **β-glucan**
- **N-linked mannans**
- **C-type lectin receptors**

**Toll-Like Receptors**
- TLR2
- TLR4
- MyD88
- NF-κB activation
- CARD9

**Co-stimulatory molecules**
- IL1β, Pro-IL1β
- Th17 priming cytokines

**Naïve T cells**
- IL1β, IL6, IL23, TGFβ
- STAT1
- STAT3

**Th17 cells**
- IL17
- IL22
- Reactive oxygen species
- Chemotaxis

**Th17 priming cytokines**
- IL17A/F
- CCR4 / CCR6

**Cytokines**
- IL1β, IL6, IL23, TGFβ
- Pro-IL1β
- Th17 priming cytokines
- Co-stimulatory molecules

**Naïve T cells**
- STAT1
- STAT3

**Th17 cells**
- IL17
- IL22
Fungal PAMPs

O-linked mannans, Zymosan

Toll-Like Receptors

β-glucan

AR Dectin1 deficiency

N-linked mannans

C-type lectin receptors

Co-stimulatory molecules

Th17 priming cytokines

IL1β, IL6, IL23, TGFβ

Gain-of-Function STAT1

N-linked mannans

AR CARD9 deficiency

AR IL17RA deficiency

CCR4 / CCR6 → Chemotaxis

Anti-IL17 Autoantibody (APECED)

IL22 and Inflammatory cytokines

Naïve T cells

Th17 cells

IL17A/F

Gain-of-Function STAT3

STAT1

STAT1

Job syndrome

IL1β, IL6, IL23, TGFβ

Pro-IL1β

IL1β

AR Dectin1 deficiency

AR CARD9 deficiency

AR IL17RA deficiency

STAT1

Dominant neg.

DC-SIGN

MINCLE

Mannose R

Dectin1*

Dectin2

MyD88

NF-κB activation

CARD9*

Dectin1

Pro-IL1β

Th17 priming cytokines

Gain-of-Function STAT3

STAT1

Th17 cells

Naïve T cells

Sam AIRE*
Mendelian susceptibility to mycobacterial disease

- Predisposition of otherwise apparently healthy individuals to infections caused by weakly virulent mycobacteria (BCG) and atypical (or nontuberculous) mycobacteria, *M. tuberculosis* and Salmonella

- Genes:
  - IFNGR1 (AR/AD)
  - IFNGR2 (AR)
  - STAT1 (AD)
  - IL12B (AR)
  - IL12RB1 (AR)*
  - NEMO (IKBKG) (XL)
  - CYBB (XL)
  - IRF8 (AR/AD)
  - ISG15 (AR)
  - (TYK2 – AR)

→ Impaired IFN-γ-mediated immunity (~50 % have unknown genetics)
Mycobacteria (Salmonella)  

**NF-κB activation**  

Phagocytosis  

**IL12 (IL12B*)**  

**Cybb (GP91-PHOX)** *  

**ISG15***  

**HLA-II**  

**Antigen presentation**  

**HLA- II**  

**IL12 (IL12B*)**  

**Macrophage**  

**Dendritic cell**  

**Granulocyte (WBCs)**
Mycobacteria (Salmonella)

Phagocytosis

NF-κB activation

NEMO*

Antigen presentation

IL12 (IL12B*)

TYK2*

Helper T

Cytotoxic T

HLA-II

TCR

IL12

IFNγ

IFNGR1*

IFNGR2*

ISG15*

CYBB (GP91-PHOX) *

Dendritic cell

Macrophage

Granulocyte (WBCs)

HLA-III

IRF8*

IFNγ

IL12R*

STAT1*

IFNγ

ISGs

NK cells (others)

Mycobacteria

Phagocytosis

CYBB (GP91-PHOX) *
**NF-kB activation**

**Antigen presentation**

**IL12 (IL12B*)**

**TYK2***

**Helper T**

**Cytotoxic T**

**Mycobacteria (Salmonella)**

**Phagocytosis**

**CYBB (GP91-PHOX) ***

**XL CYBB deficiency**

**Granulocyte (WBCs)**

**ISG15***

**AR IL12B deficiency**

**AR ISG15 deficiency**

**AR STAT1 deficiency**

**AR IRF8 deficiency**

**AR IL12R deficiency**

**AR TYK2 deficiency**

**AR IFNGR2 deficiency**

**AR IRF8 deficiency**

**AR IL12R deficiency**

**XL NEMO deficiency**

**AR/AD IFNGR1 deficiency**

**IFNγ**

**NK cells (others)**

**Cytotoxic T**

**Helper T**

**TDN**

**NEMO***
Herpes Simplex Encephalitis

- HSE is the most common cause of sporadic fatal encephalitis worldwide (70% mortality if untreated)
- Acyclovir
- Lifelong neurological sequelae of varying severity in survivors is common (>30%)
- Genes:
  - TLR3 (AR/AD)
  - UNC-93B (AR)
  - TRIF (AR/AD)
  - TRAF3 (AD)
  - TBK1 (AD)
  - NEMO (XR)
  - STAT1 (AR)
  - TYK2 (AR)
Herpes Simplex Encephalitis

**Endosome**

- **ER**
- **dsRNA**
- **TLR3**
- **UNC93B**
- **TRIF**
- **TRAF3**
- **TBK1**
- **NEMO**
- **NF-κB activation**
- **Type I/III IFN**

**AR/AD TLR3 deficiency**

- **AR UNC93B def.**
- **AR/AD TRIF def.**

**AD TRA\(F\) def.**

**AD TBK1 def.**

**XD NEMO def.**

**RNaseL, PKR**

**AR STAT1 def.**

**AR TYK2 def.**

**Interferon Response Genes**

- **STAT1**
- **IFNAR1**
- **IFNAR2**
- **IFNLR1**
- **IL10RB**
- **IFNα/β**
- **ISRE**

**Interferon Response**

- **IFNα**

**Type I/III IFN**
Different genes – same/similar clinical phenotype

• CMC:
  - IL-17RA, IL-17F, STAT1, DECTIN1, CARD9, STAT3, DOCK8, AIRE

• MSMD:
  - IFNGR1, IFNGR2, STAT1, IL12B, IL12RB1, NEMO, CYBB, IRF8, ISG15, TYK2

• HSE:
  - TLR3, UNC-93B, TRIF, TRAF3, TBK1, NEMO, STAT1

→ Molecular networks/pathways, not genes
→ Single gene testing vs. gene panels
One gene – multiple phenotypes

• **AD STAT1** deficiency: *dominant negative* mutations
  → susceptibility to mycobacterial and salmonella infections

• **AD STAT1** deficiency: *gain-of-function* mutations
  → chronic mucocutaneous candidiasis

• **AR STAT1** deficiency: *loss-of-function* mutations
  → susceptibility to severe viral and mycobacterial disease
Definition of PIDs

• defined based on clinical/immunological phenotype

• defined based on the molecular defect

→ none of these definitions are perfect
Genetics of PIDs

- Manifestations (infections) can be the same in both primary and secondary ID as well in “healthy” individuals
  - Genetics + Environment
- ~150 PIDs, >200 genes (both growing)
  - increasing role for mutation testing (PIDs defined by genes)
  - novel technologies (whole genome/exome)
- One disease multiple genes (overlapping phenotypes)
  - CMC, MSMD, HSE
- One gene, multiple diseases
  - STAT1, NEMO
- Inheritance: AR, AD, XL
  - AR/AD IFNGR1
- Molecular networks/pathways, not genes
  - recognition → signaling → effector function
References

• Primary immunodeficiencies

• Chronic mucocutaneous candidiasis

• Mendelian susceptibility to mycobacterial disease
  - de Beaucoudrey L et al. Medicine 89:381-402, 2010

• Genetic susceptibility to herpes simplex encephalitis