Genetic infectious susceptibility and TLR defects in human

Bacteria

Mycobacteria

Herpes simplex virus

Human Genetics of Infectious Diseases-INSERM U980,
Necker Faculty, Paris Descartes University, Paris, France

capucine.picard@inserm.fr
Toll-like receptors (TLR)

- Receptor of innate immunity (11 in human)
- Membrane glycoproteines (90-115 kD)
- Leucin rich repeat (LRR)
- Recognize ligands derived from microorganisms: LPS – TLR4 or dsRNA-TLR3
- Initiation of inflammatory response

(Bell et al. TRENDS in Immunology 2003)
Toll like receptors and IL-1Rs (TIRs) signalling pathway

(Picard et al. 2011 in press Clinical Microbiology Reviews)
Inherited human IRAK-4 and MyD88 deficiencies

IL-1Rs → TLRs → TIR Domain

MyD88 → IRAK4 → IKK (α, β) → IκBα

NF-κB (p50/p50, p50/p65) → MAPK

Gene transcription:
IL-1β, IL-6, IL-12, TNF-α, IFN-γ...

(map from Picard et al. Science 2003)
(map from von Bernuth et al. Science 2008)
Inherited human IRAK-4 and MyD88 deficiencies

Blood cells of IRAK-4 and MyD88 deficient patients display an impaired responses to TLRs ligands (except TLR3) and IL-1β

IRAK-4 deficiency – patients

52 patients from 33 kindreds in 14 countries
(Autosomal recessive disorder)
MyD88 deficiency (AR) – patients

von Bernuth et al. 2008 Science
Conway et al. 2010 JACI
Picard et al. 2010 Medicine
Unpublished datas

22 patients from 7 kindreds in 6 countries
Patients are homozygous or compound heterozygous for mutations in \textit{IRAKe4}.

Patients are homozygous or compound heterozygous for mutations in \textit{MyD88}.
IRAK-4/MyD88 deficiencies (n=61)

Clinical manifestations by patient

- Pneumonia
- ENT infections
- Skin abscess / orbital cellulitis
- Lymphadenitis
- Deep inner organ/ abscess
- Arthritis / osteomyelitis
- Sepsis
- Meningitis

Patients (%)

MyD88 deficient patients
IRAK-4 deficient patients
IRAK-4/MyD88 deficient pts

Invasive bacterial infection: 148 reported episodes of InvBD
(n=2.4 episodes/patient. range: 0 to 10)
**IRAK-4/MyD88 deficiencies: bacterial infections**

**Invasive infections**

- **IRAK-4 deficient pts**
  - 7.6% S. pneumoniae
  - 18.1% S. aureus
  - 5.7% Other Gram-pos
  - 14.3% Other Gram-neg

- **MyD88 deficient pts**
  - 14.7% S. pneumoniae
  - 14.7% S. aureus
  - 5.9% Other Gram-pos
  - 20.6% Other Gram-neg

**Non invasive infections**

- **IRAK-4 deficient pts**
  - 8.1% S. pneumoniae
  - 1.6% S. aureus
  - 22.6% Other Gram-pos
  - 16.1% Other Gram-neg

- **MyD88 deficient pts**
  - 13.3% S. pneumoniae
  - 13.3% S. aureus
  - 20% Other Gram-pos
  - 53.3% Other Gram-neg

**S. pneumoniae** = 52% of invasive bacterial infections
(Meningitis. sepsis. arthritis. osteomyelitis. deep abscess)

**S. aureus** = 45.5% of non invasive bacterial infections
(Cellulitis. omphalitis. sinusitis. ENT infections. pneumonia)

S. pneumoniae isolated in 41 pts / 61 (IPD 67%)
IRAK-4/MyD88 deficiencies: severe, narrow and transient phenotype

**Early clinical phenotype:**
1st invasive infection occurred < 2 years

**Severe clinical phenotype:**
25 patients died of invasive bacterial infections
(16 caused by invasive bacterial *S. pneumoniae* infection)

**Transient clinical phenotype:**
no severe invasive infections > 14 years
no deaths after the age of 8 years.
Immunological explorations

- Blood lymphocyte and monocyte subsets: normal
- T cell proliferations (mitogens and antigens): normal
- Complement pathways: normal
- Ig: normal, except high levels IgG4 (35%) and IgE (65%)
- Abs response to proteins: normal
- Abs response to polysaccharides: variable
- Inflammatory signs (clinical & biological): low
IRAK-4/MyD88 deficiencies - summary

- Invasive infections by Gram-positive bacteria, but also Gram-negative
  \((S.\ pneumoniae, S.\ aureus\ and\ P.\ aeruginosa)\ <\ 14\ years\ of\ age\)
- Recurrent episodes of cellulitis \((S.\ aureus)\ and\ sinusitis\ persisting\)
- IMMUNODEFICIENCIES THAT IMPROVE WITH AGE
  - Delayed or low inflammatory signs (fever, PMN, CRP…)
  - Delayed separation of the umbilical cord \(\text{Takada.}\ 2006\ J.\ Ped\)
  - Deficient IL-1β and TLR signalling \(\text{except for TLR3}\)
  - No other susceptibility to infection, in particular severe viral infection
MyD88 and IRAK-4 deficiencies: phenocopies

8 Hours

IL1-β | TNF-α | Poly(I:C)

1451 Transcripts | Normalized Expression Level

(Fibroblast cells)
Toll like receptors and IL-1Rs (TIRs) signalling pathway

TLR-1, 2, 5, 6, 10 → IL-1Rs

MyD88 → IRAK-4, IRAK-1

TLR-7, 8, 9 → MyD88

IFN-β → IRF3

NF-κB → AP1, MAPK, IL-6, IL-1, TNFα

TRIF → TRAF3, IKKε, TBK1, IRF3

IL-6, IL-1, TNFα → AP1

IFN-β → IRF3
Herpes Simplex virus Encephalitis (HSE):
a devastating viral disease of unclear pathogenesis

HSV-1 reach the CNS via neurons and do not spread to epithelia and internal organs

Classical Primary Immunodeficiencies, do not usually predispose to HSE

HSE patients are normally resistant to most infectious agents, including neurotropic viruses

2-4 cases/million people/year

Incidence peaks 6 mos-6 yrs during primary infection by HSV-1

Up to 30% mortality in treated individuals
HSE: a genetic epidemiological survey

10% consanguinity

Number of HSE

Age in yrs

Prevalence of HSV+

100
80
60
40
20
0
10 20 30 40 50 60 70 80

HSE
HSV+

0 10 20 30 40 50 60 70 80

0 20 40 60 80 100
Autosomal Recessive UNC-93B deficiency: the first genetic etiology of isolated HSE

- UNC-93B is a 12-transmembrane domain protein in the ER.
- UNC-93B delivers the nucleotide-sensing receptors TLR3, 7, 8 and 9 from the ER to endolysosomes

Three individuals, from Portugal and France (2 gypsies)) from 2 consanguineous kindreds with homozygous mutations in *UNC93B1*:

P1 HSE at 11, 14 and 42 months
P2 HSE at 5 and 17 years
P3 asymptomatic at 30 yrs (HSV1) ⇒ incomplete penetrance

(Casrouge et al. Science 2006)
Autosomal Recessive UNC-93B deficiency: 
the first genetic etiology of isolated HSE

(Casrouge et al. Science 2006)
Autosomal Dominant TLR3 deficiency: The second genetic etiology of HSE

7 individuals bearing TLR3 AD mutations have been identified from two unrelated kindreds, 2 had HSE (incomplete penetrance)
P1 HSE at 5 years
P2 HSE at 5 months

(Zhang Science 2007)
Fibroblast cells from AD TLR3 deficient patients have impaired type 1 and 3 IFN production

(Zhang Science 2007)
Heterozygous TRAF3 mutation in a patient with HSE: 
The third genetic etiology of HSE

TRAF-3 has functions downstream from multiple TNF receptors and the receptors inducing IFN-α, IFN-β, and IFN-λ production, including TLR3.

(Schneider et al. *Nature Immunology*, 2006)
Heterozygous TRAF3 mutation in a patient with HSE

200 patients sequenced

TRAF3 c.705C>T
TRAF3 protein R118W

P1 HSE at 4 years

TRAF domain

Zinc-finger

Isoleucin zipper

RING finger

Homo sapiens  ILALQVYCRNESRGSCAQTLGLGH
Pan troglodytes  ILALQVYCRNESRGSCAQTLGLGH
Macaca mulatta  ILALQVYCRNESRGSCAQTLGLGH
Equus caballus  ILALQVYCRNESRGSCAQTLGLGH
Monodelphis domestica  ILALQVYCRNESRGSCAQTLGLGH
Ornithorhynchus anat  ILALQVYCRNESRGSCAQTLGLGH
Mus musculus  ILALQVYCRNESRGSCAQTLGLGH
Sus scrofa  ILALQVYCRNESRGSCAQTLGLGH
Rattus norvegicus  ILALQVYCRNESRGSCAQTLGLGH
Bos taurus  ILALQVYCRNESRGSCAQTLGLGH
Canis familiaris  ILALQVYCRNESRGSCAQTLGLGH
Gallus gallus  ILALQVYCRNESRGSCAQTLGLGH
Onychorhynchus mykiss  ILALQVYCRNESRGSCAQTLGLGH
Danio rerio  ILALQVYCRNESRGSCAQTLGLGH

(Rebeca Pérez de Diego et al. Immunity 2010)
SV40 fibroblast studies: IFN type I and cytokines production

(Rebeca Pérez de Diego et al. Immunity 2010)
SV40 fibroblast studies: Molecular phenotype

NF-κB pathway

IRF3 dimerization

Fibroblast cells from TRAF3 deficient pt:
Reduced NF-kB nuclear translocation and IRF3 dimerization in response to TLR3 agonist

(Rebeca Pérez de Diego et al. Immunity 2010)
Complementation studies in SV40 fibroblast of P1

Stable transfectants

(Rebeca Pérez de Diego et al. Immunity 2010)
TRAF3 in other pathways

TLR4 and TLR7/8

Impaired IFN and cytokines production by MDDC and MDM

Monocyte derived dendritic cells (MDDC)
Monocyte differentiated macrophages (MDM)

(Rebeca Pérez de Diego et al. Immunity 2010)
Impaired IL-6 production after CD40L activation by MDDC

Diminished the production of IL-8 by SV40-fibroblast

Constitutive activation in B-EBV cells - IL10 production

**CD40**

**LTβR**

**BAFFR**

**TRAF3 in other pathways**

*(alternative NF-kB pathway)*
Negative dominant effect
Human AD TRAF3 deficiency

• Heterozygous R118W mutation in TRAF3
  ➔ dominant-negative effect and results in impaired TLR3-dependent induction of IFN and predisposition to HSE

• TRAF3 patient displays a broad cellular phenotype:
  – with impaired cellular responses to IFN TLR3-independent pathways,
  – CD40, LTβR and BAFFR, whose clinical consequences have so far remained silent.
**HSE-Specific Immunity**

**Production of type I IFNs**
- TLR3
- UNC93B
- dsRNA
- ssRNA
- CpG
- TLR7/8/9
- MYD88
- IRAK4
- IKK complex
- IRF3
- IRF7
- NFκB
- ISGF3 complex
- STAT2
- IRF9
- HSV-1

**Response to type I IFNs**
- TLR3
- TRIF
- TRAF3
- UNC93B
- NEMO
- IFN-αR1
- IFN-αR2
- JAK1
- TYK2
- STAT1
- STAT2
- IRF9
- ISGF3 complex
- ISRE genes

**Pathways**
- dsRNA
- ssRNA
- CpG
- TLR3
- TRIF
- TRAF3
- UNC93B
- NEMO
- IFN-αR1
- IFN-αR2
- JAK1
- TYK2
- STAT1
- STAT2
- IRF9
- ISGF3 complex
- ISRE genes
Genetic infectious susceptibility and TLR defects in human
Conclusions

• The TIRs signaling pathway (IRAK-4/MyD88-depend pathway) is crucial to control invasive pyogenic bacterial infection during childhood, by induction and propagation inflammation, and secondary initiation of adaptive immunity.

• The integrity of the TLR3-IFN pathway is crucial to control HSV-1 primary infection in the CNS.

• The exploration of « idiopathic infections » could contribute to diagnose new primary immunodeficiencies and to a better understanding of immunity against pathogens.
Acknowledgements

IRAK4 and MyD88 collaborators

HGID-INSERM-U980
Anne Puel
Horst von Bernuth.
Pegah Ghandil.
Mayah Chrabieh.
Cheng-Lung Ku.
Laurent Abel
Jean-Laurent Casanova

UK:
Rainer Doffinger. D Kumararatne
Helen Chapel. Graham Davies
Peter Arkwright. Adrian Thrasher
Claire Bethune

Canada:
David Speert. Andrew C. Issekutz
Chaim Roifman

Australia:
Mimi Tang. Joanne Smart

Israel:
Ben-Zion Garty

Turkey:
Yildiz Camcioglu

Spain:
Carlos Rodriguez-Gallego
Carlos Rodrigo, Maria Méndez
Francisco Almazán, Claudia Fortuny
Laia Alsina, Elena Colino,
Juan Ignacio Aróstegui

Germany:
Stephan Ehl

Switzerland:
Janine Reichenbach

Slovenia:
Simona Eva Zitnik

France:
Cyrille Hoarau
Nicolas Sirvent, Dominique de Ricaud
Francois Dubos, Frédérique Delion

Hungary:
László Maródi

Japan:
Hidetoshi Takada.
Toshiro Hara
Hideto Yoshikawa
Shigeaki Nonoyama

KSA:
Sami Al-Hajjar
Abdulaziz Al-Ghonaium
Saleh Al-Mohsen

USA:
Noorbibi K. Day-Good
Steven M. Holland. John Gallin.
Jens C. Krause. Clarence B Creech.
Coleen K. Cunningham. Joseph Domachowske
Ofer Levy. Douglas McDonald. Raif Geha

Portugal:
Artur Bonito Vitor, Júlia Vasconcelos
Margarida Guedes

Spain:
Arturo Bonito Vitor, Júlia Vasconcelos
Margarida Guedes
Acknowledgements

HSE

TLR3

HGID Rockefeller Branch
Shen-Ying Zhang
Emmanuelle Jouanguy
Yiqi Guo
Melina Herman
Jean-Laurent Casanova

HGID Necker Branch
Vanessa Sancho Shimizu
Lazaro Lorenzo-Diaz
Rebeca Perez
Annabelle Cardon
Soraya Boucherit
Anne Puel
S Plancoulaine
Rebeca Pérez de Diego
Laurent Abel,

Institut Pasteur
Lluis Quintana

iFREC Osaka University
Osamu Takeuchi

UCLA
Sang Hoon Rhee

Cochin
Pierre Lebon,
Flore Rozenberg,

KB
Marc Tardieu,
Children and their families