

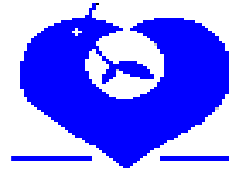
# **Genetic Factors Governing Susceptibilities to Severe Infections**

**GSK-Chair of Infectious Diseases**

**Pr Jean-Paul MIRA**



**Inserm**



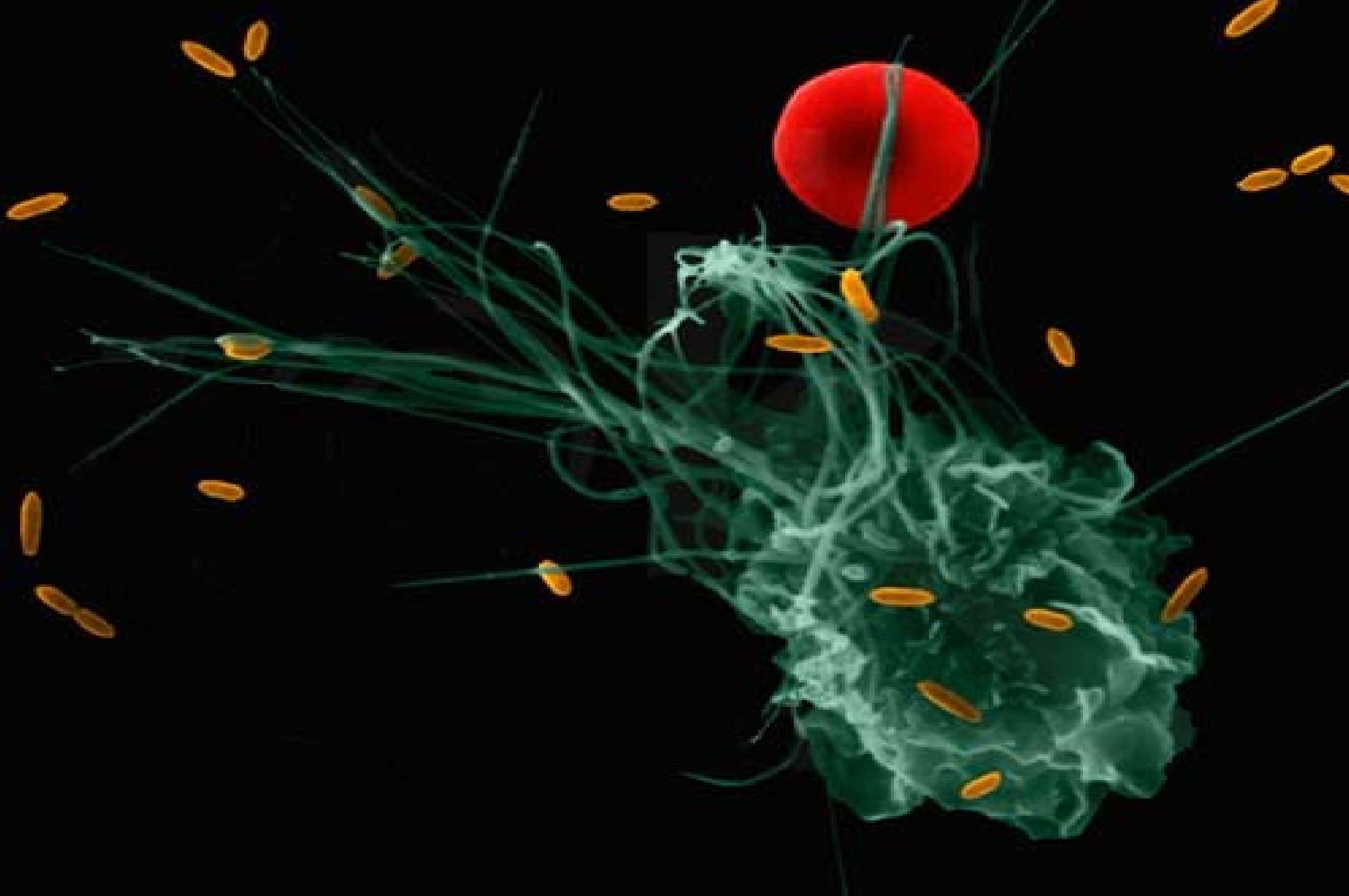
# **Polymorphisms of Toll Like Receptors and Variability of Innate Immunity**

**Institute of Cellular Pathology  
February 16, 2005**

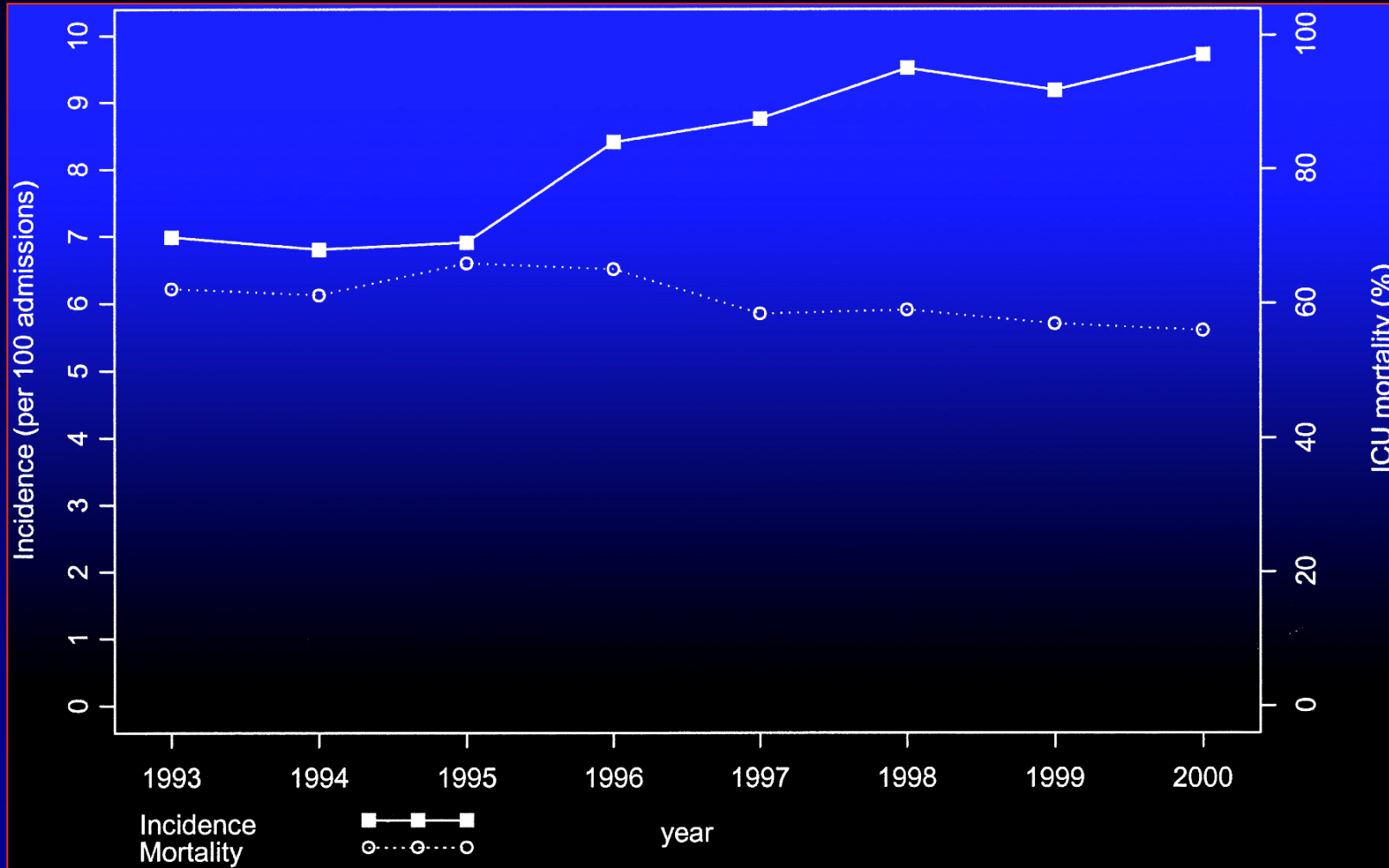
**Pr. Jean-Paul MIRA**

**Medical ICU & Dept. of Cell Biology**

**Cochin University Hospital & Cochin Institute, Paris, F**



# Septic Shock Epidemiology



# Variability of Severe Sepsis Phenotype



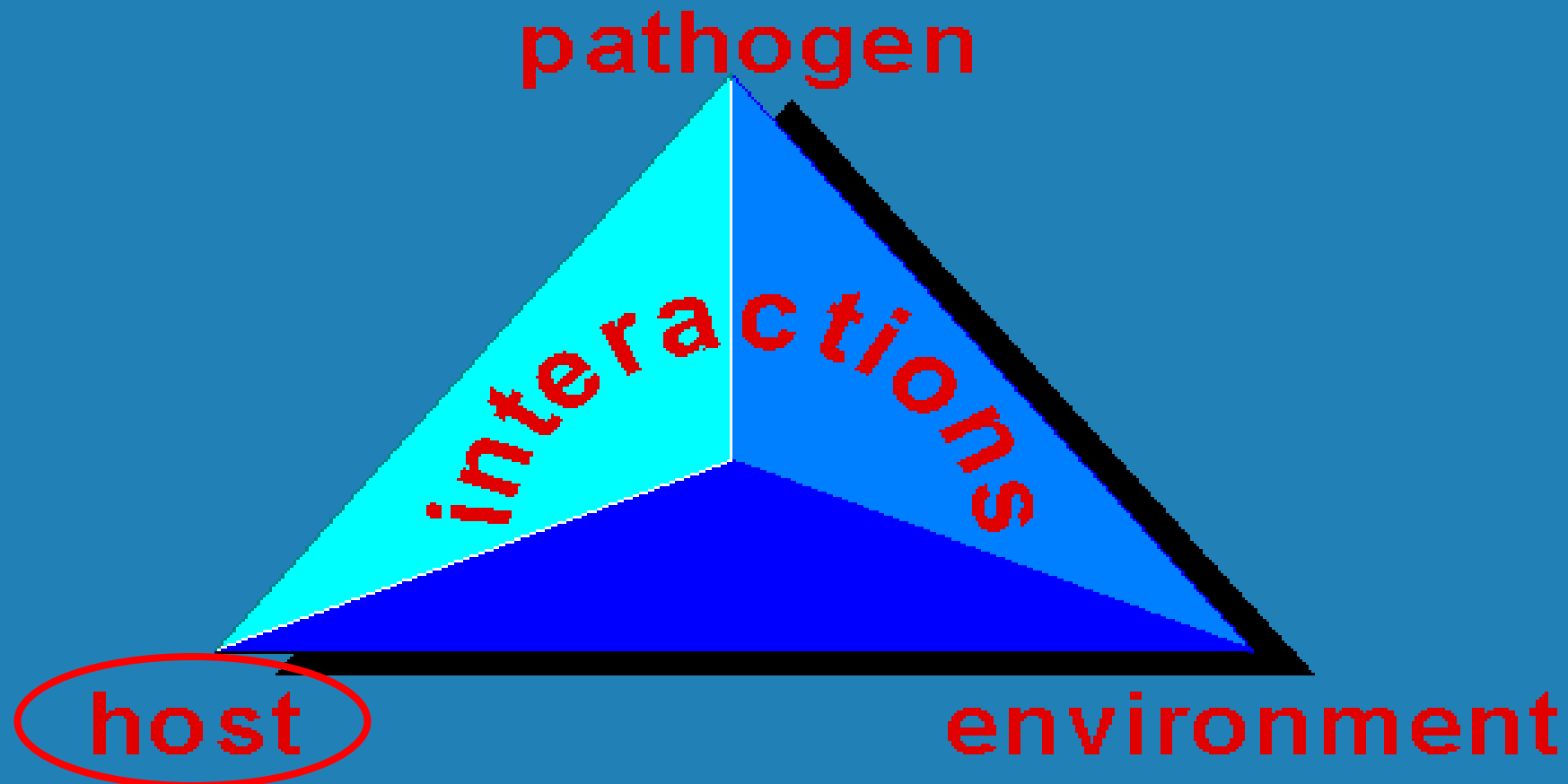
**MIEUX COMPRENDRE**

**POUR MIEUX GUERIR**

# Disease Triangle

---

$P$  (illness) =  $f$  (host, pathogen, environment, interactions)

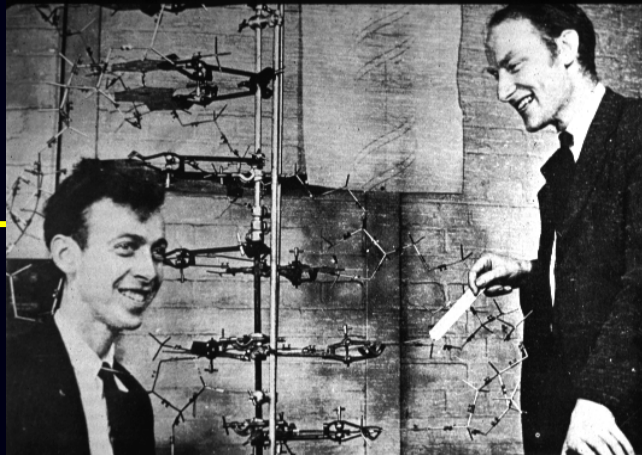




« If it were not for the great variability among individuals medicine might as well be a science and not an art »

*Sir William Osler, 1892*

-1953-



2001-2003 →



« Today we are learning the language in which God created life. It will revolutionize the diagnosis, prevention and treatment of most, if not all human diseases. »

*William J. Clinton, June 26, 2000*





**AS HUMANS, WE ALL SHARE  
THE SAME BASIC GENES**

**BUT...**



**Very small differences in genotype make beautiful differences in phenotype...**



**Small differences in genotype make big differences to phenotype**



# Evidences for a genetic component to sepsis

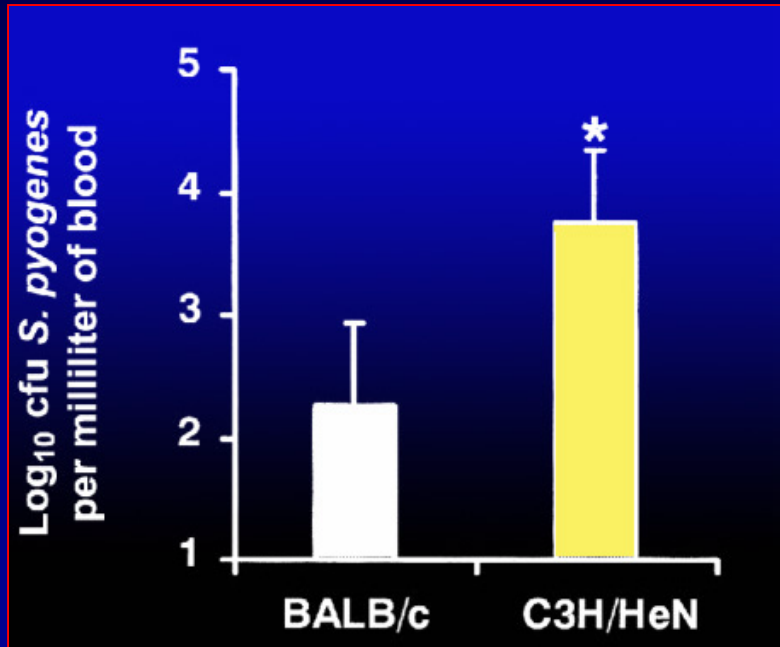
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## Animal Studies

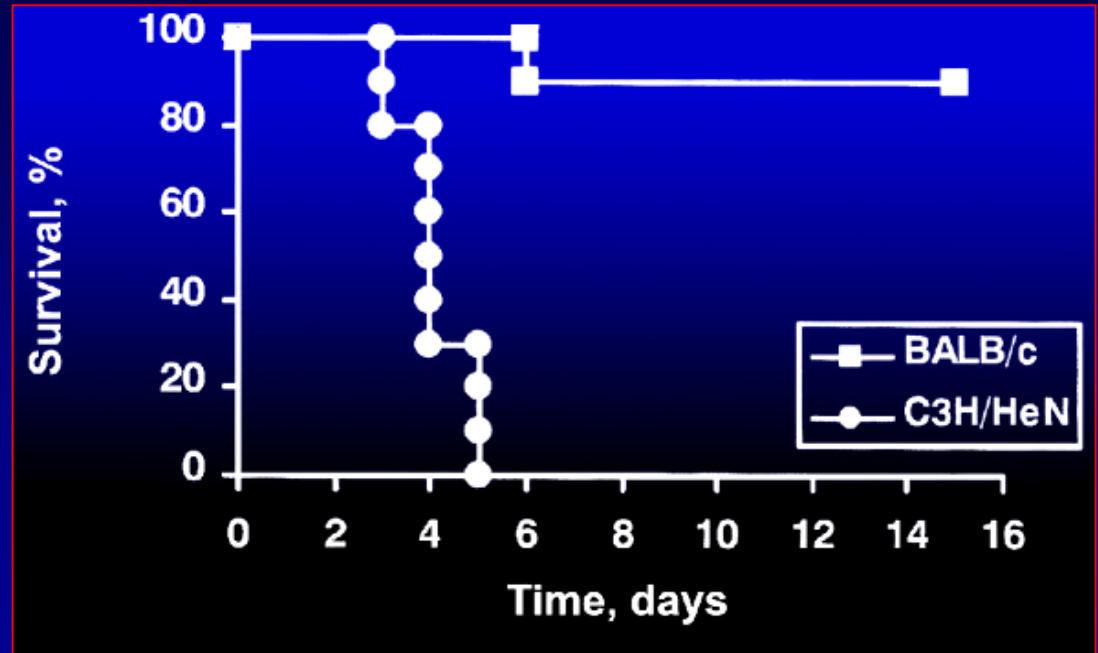
- Susceptibility/resistance to certain infection in mice

# Mice Susceptibility to Infection with Group A Streptococci

$10^3$  cfu *Strepto* Subcutaneous



d2



# Evidences for a genetic component to sepsis

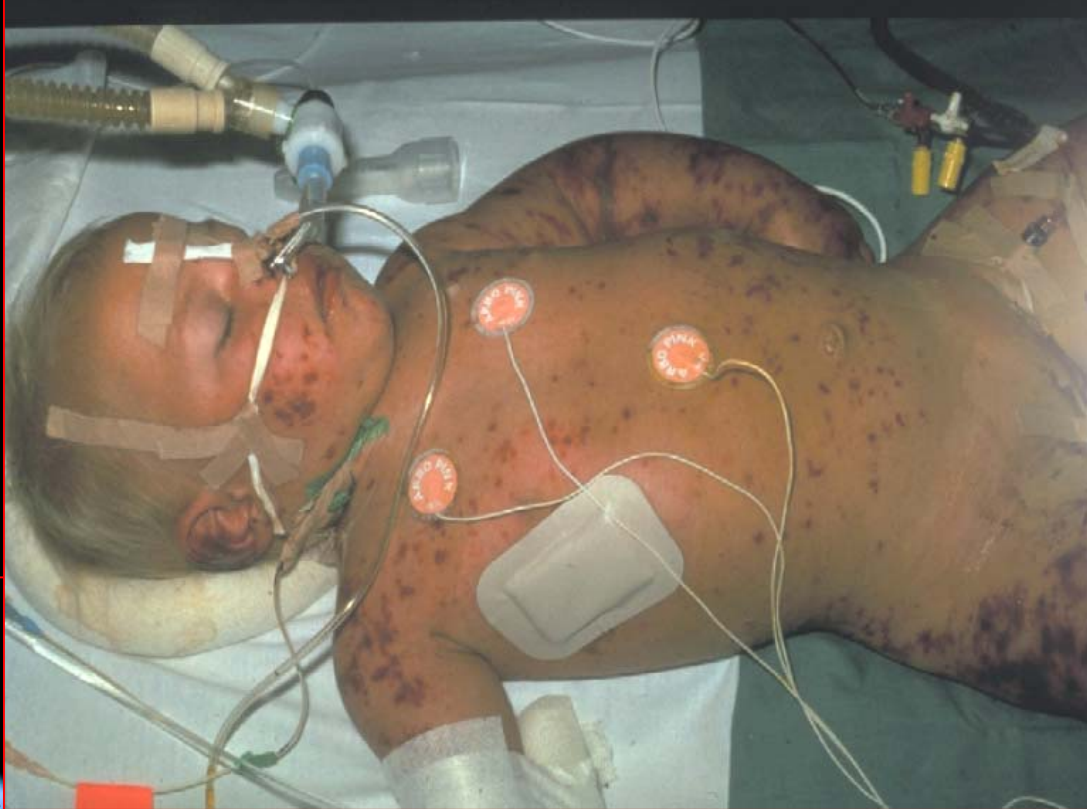
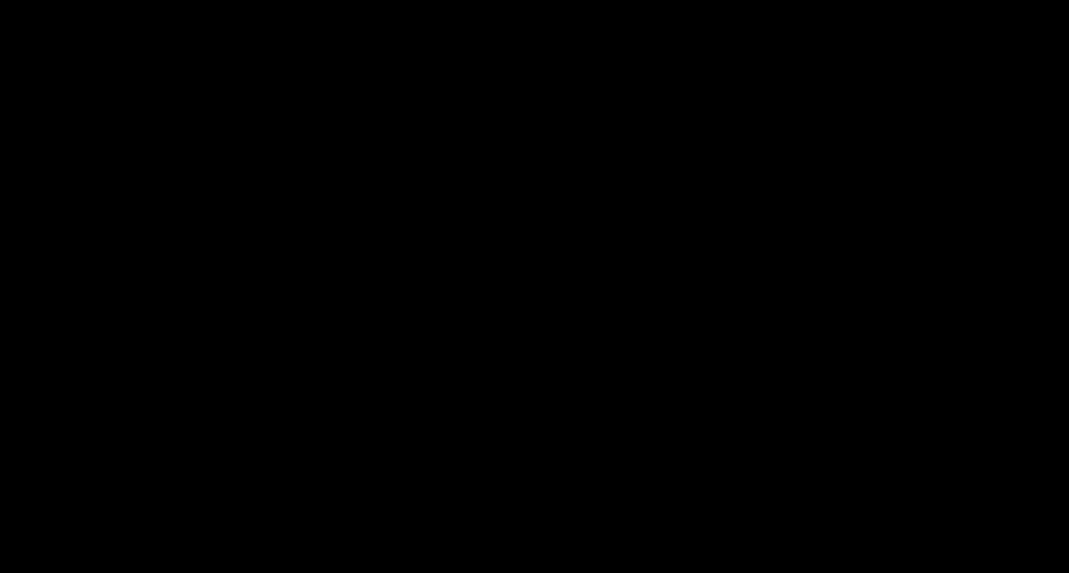
---

## Animal Studies

- Susceptibility/resistance to certain infection in mice

## Human Studies

- Clinical Evidences
- Ethnic Differences
- Twin Studies
- Adoptee Studies



# Genetic and environmental influences on premature death in adult adoptees

Cause of Death (Parent Dead before the age of 50)	Relative risk for the adoptee to die from the same cause
All causes	
Biologic	1.71
Adoptive	0.71
<b>Infection</b>	
<b>Biologic</b>	<b>5.8</b>
<b>Adoptive</b>	<b>0.73</b>
Vascular	
Biologic	4.5
Adoptive	3.1



# STUDY NATURE AND OBJECTIVES

## STUDY METHODS

**Large, observational**  
(characterizing genetic risk  
and clinical usefulness)

Population based associations

Genetic association with  
clinical outcomes

Case control cohort  
comparisons

Familial studies

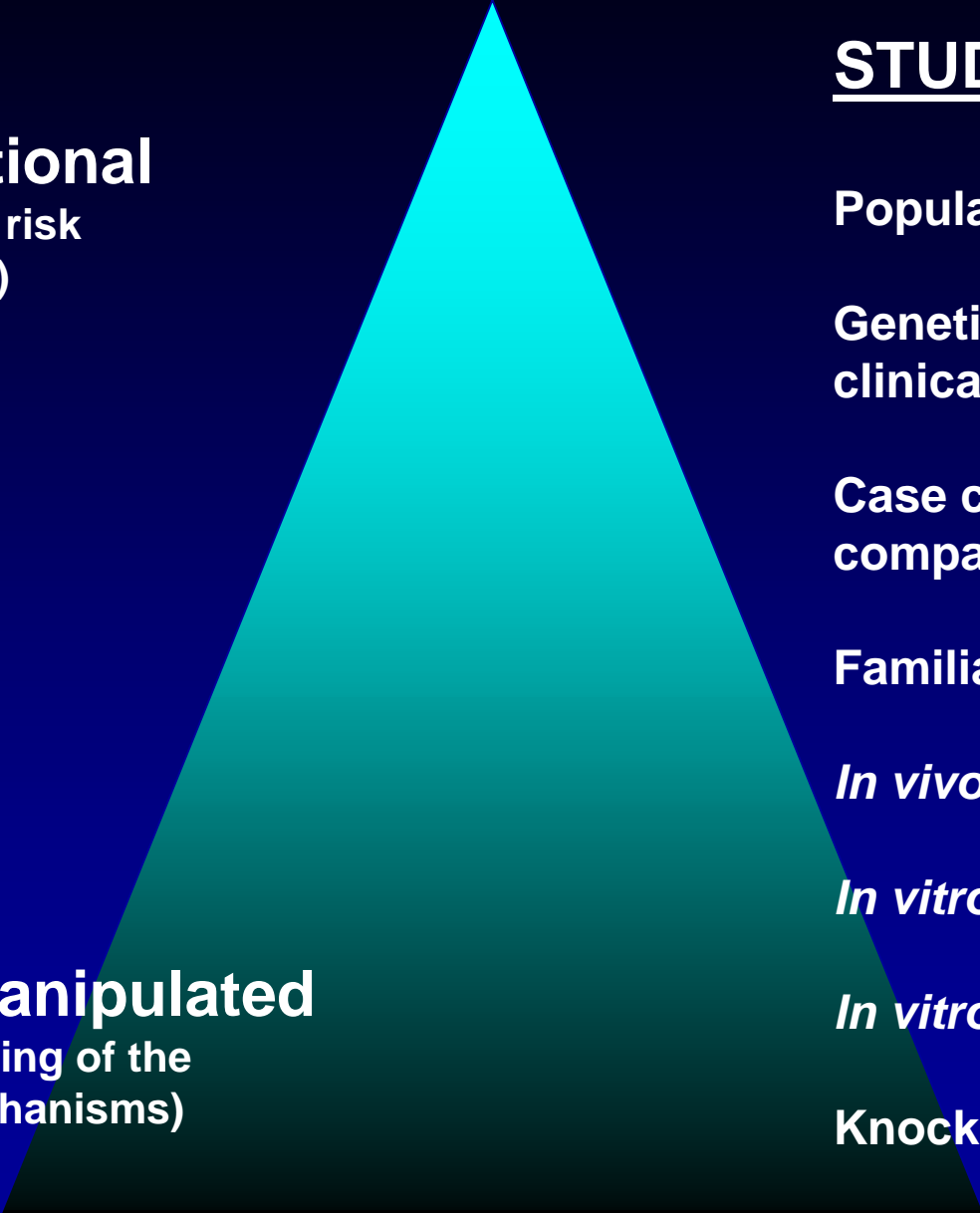
*In vivo* physiologic responses

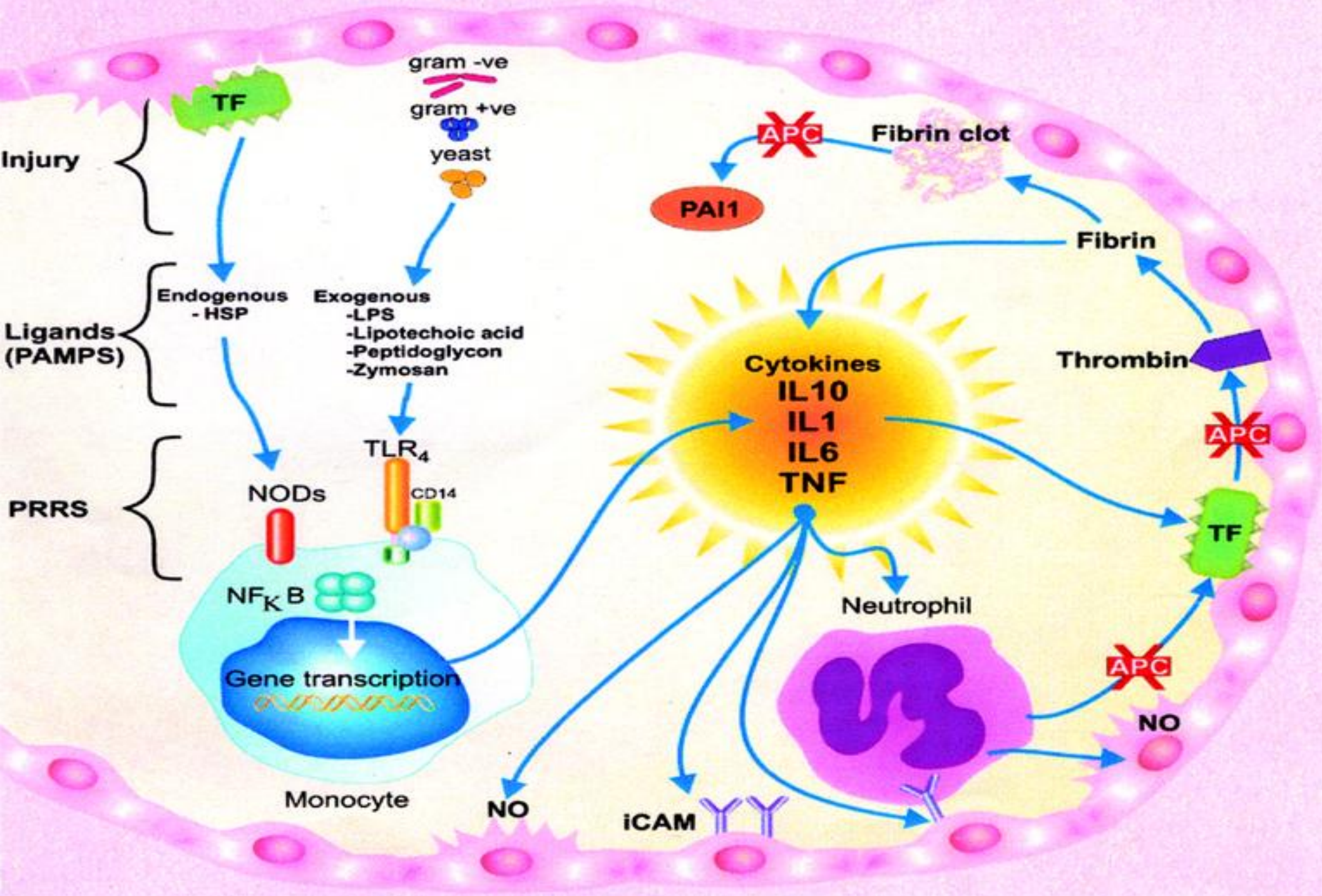
*In vitro* responsiveness

*In vitro* transfection

Knockout models

**Small, highly manipulated**  
(enhancing understanding of the  
biology of disease mechanisms)

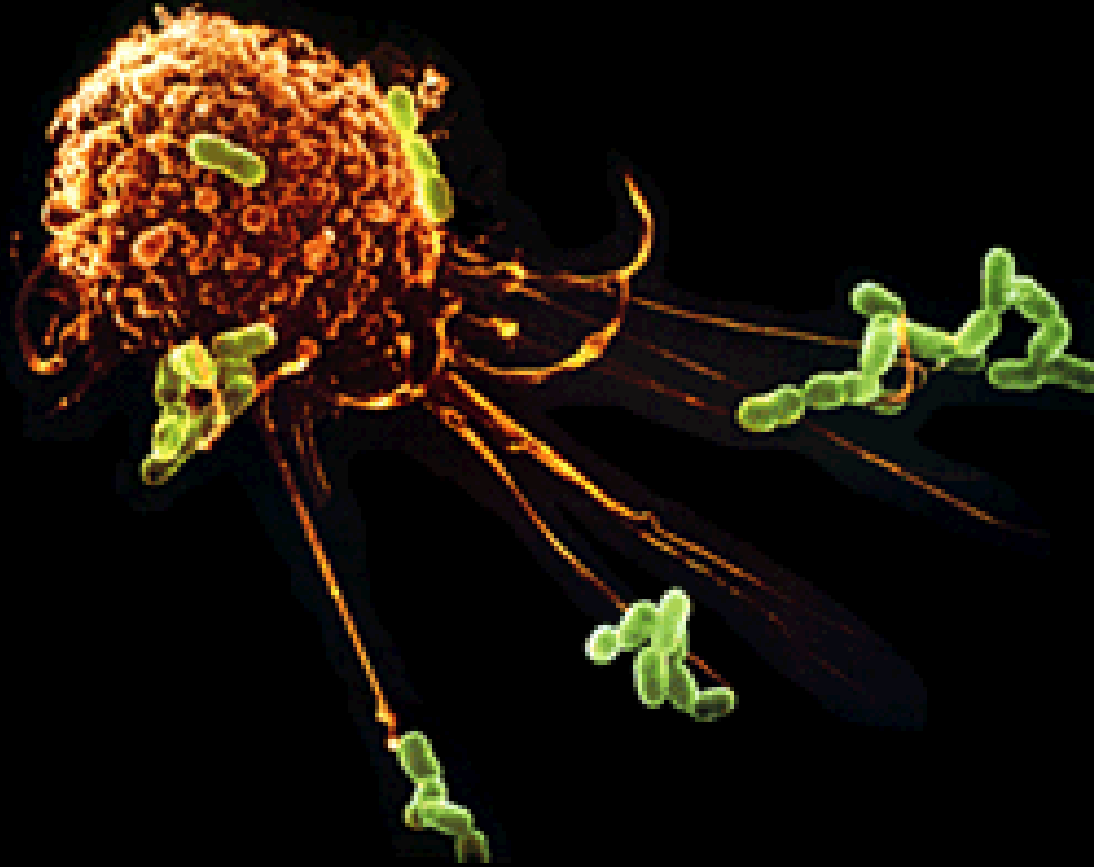


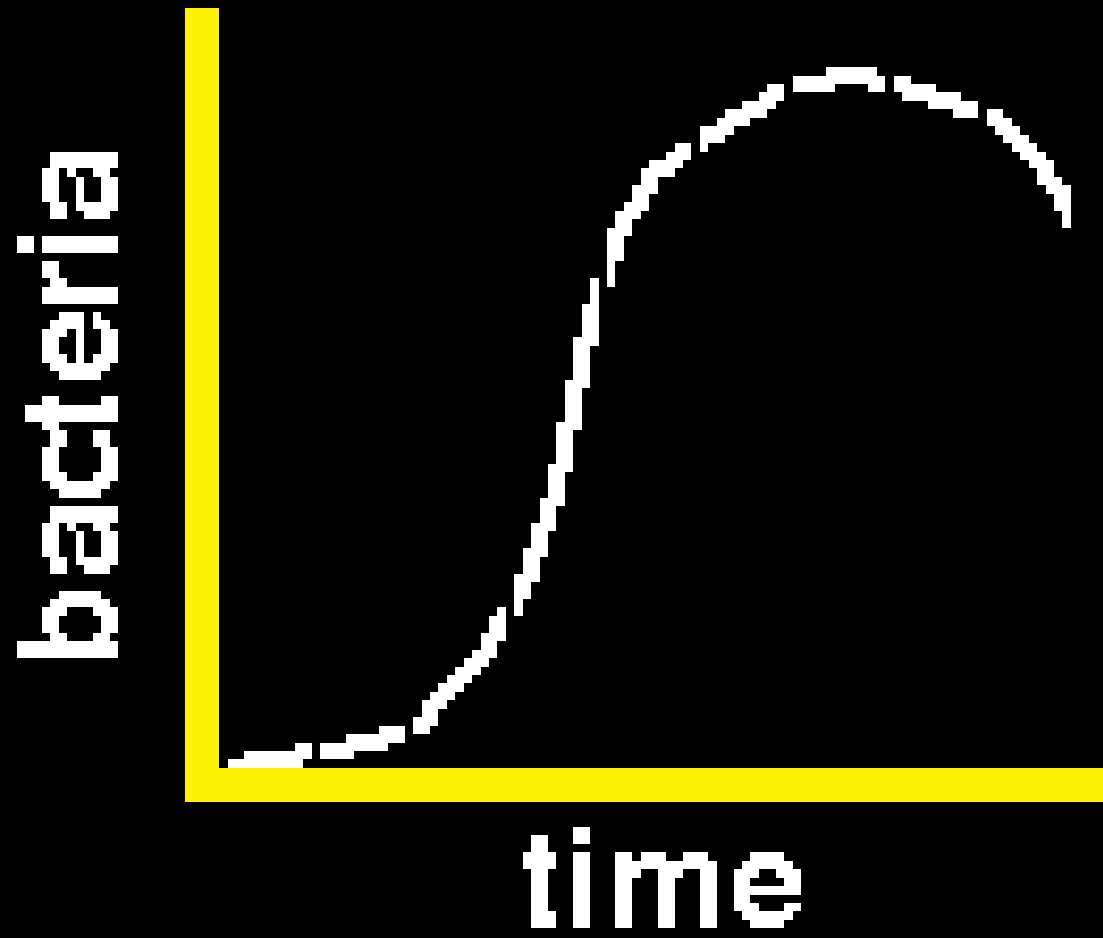


# Genetic Polymorphisms and Severe Sepsis

<b>Gene</b>	<b>Susceptibility and/or Outcome</b>
<b>Mannose Binding Lectin</b>	<b>Meningococemia, Pneumococemia Severe sepsis</b>
<b>Toll-Like Receptor 4/2</b>	<b>Gram negative/positive Septic Shock</b>
<b>Toll-Like Receptor 5</b>	<b>Legionnaire's Disease</b>
<b>CD14</b>	<b>Septic Shock</b>
<b>FC<math>\gamma</math>RII Receptor</b>	<b>Meningococemia; Pneumococemia</b>
<b>TNF locus</b>	<b>Meningococemia; Septic Shock; Malaria</b>
<b>IL-18</b>	<b>Severe Sepsis</b>
<b>IL-10</b>	<b>Severe Sepsis, Meningococemia</b>
<b>IL-6</b>	<b>Severe sepsis</b>
<b>IL-1 locus</b>	<b>Severe Sepsis</b>
<b>IL-4</b>	<b>Viral Pneumonia</b>
<b>Caspase12</b>	<b>Septic shock</b>
<b>PAI-1</b>	<b>Meningococemia; Severe sepsis</b>
<b>Factor V Leiden</b>	<b>Meningococemia; Severe sepsis</b>

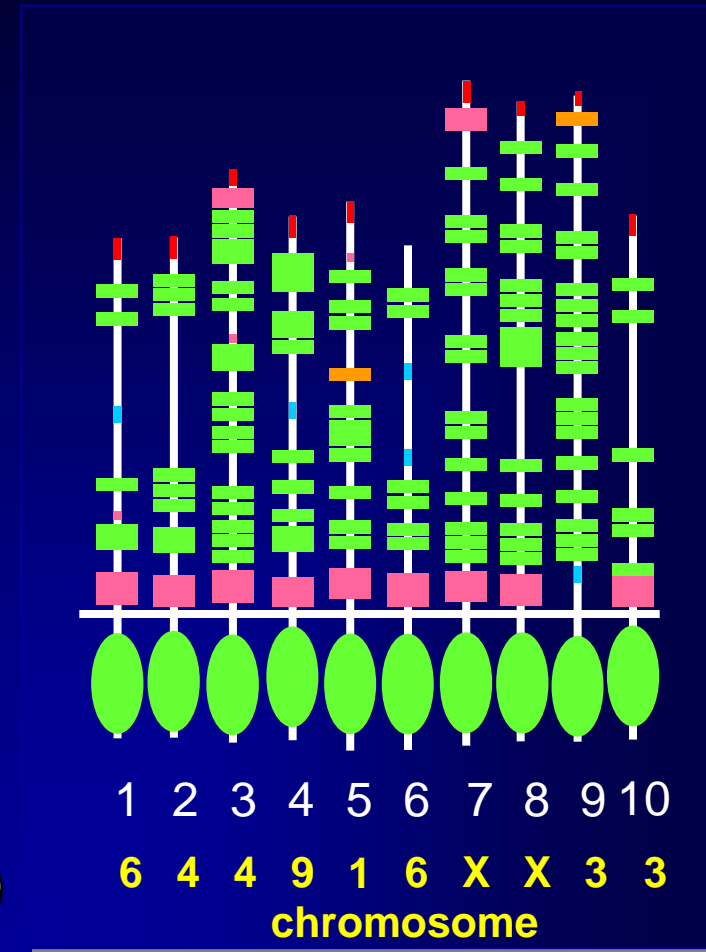
# Pathogen Detection



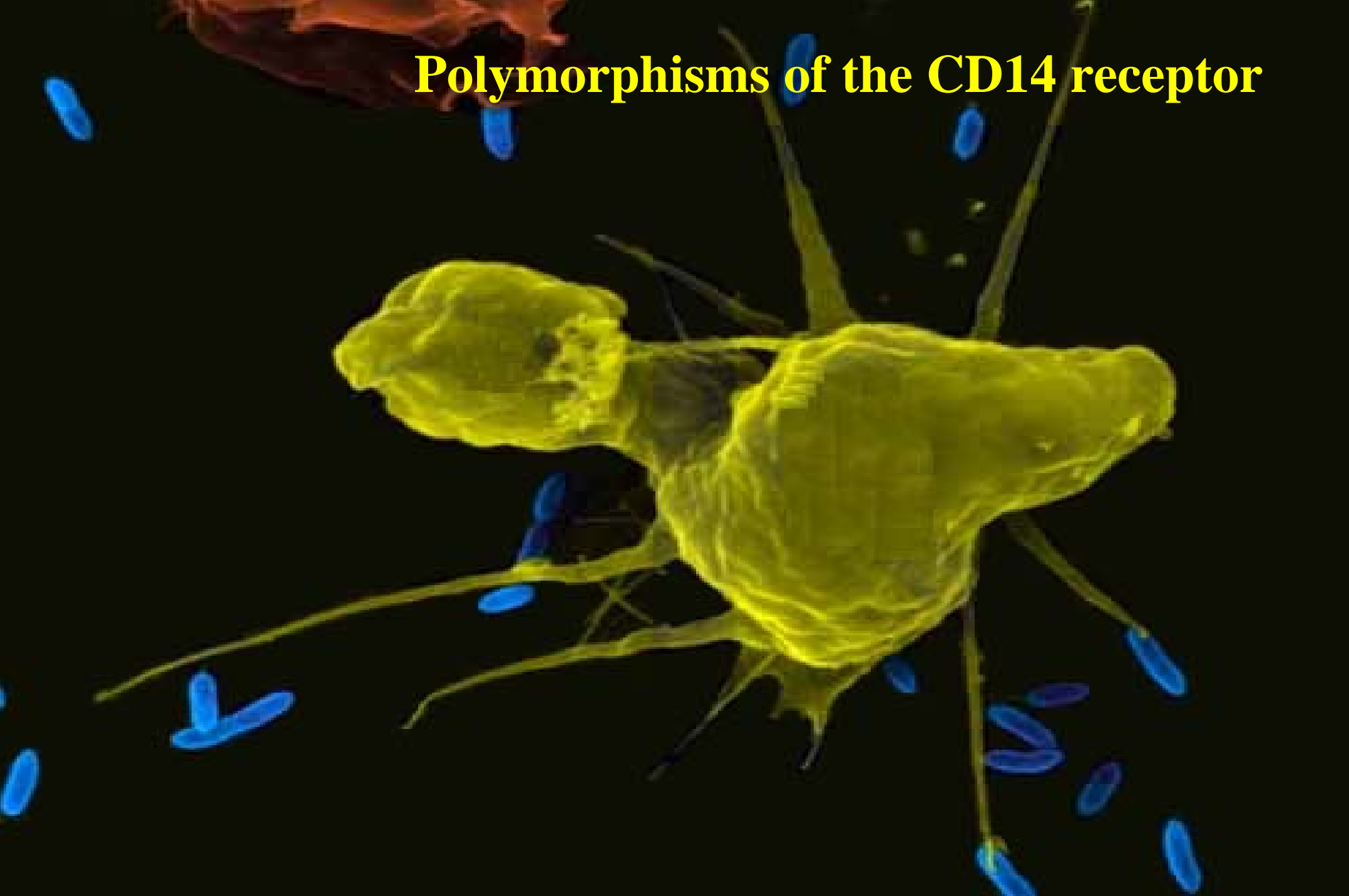


# Human Toll-like Receptors

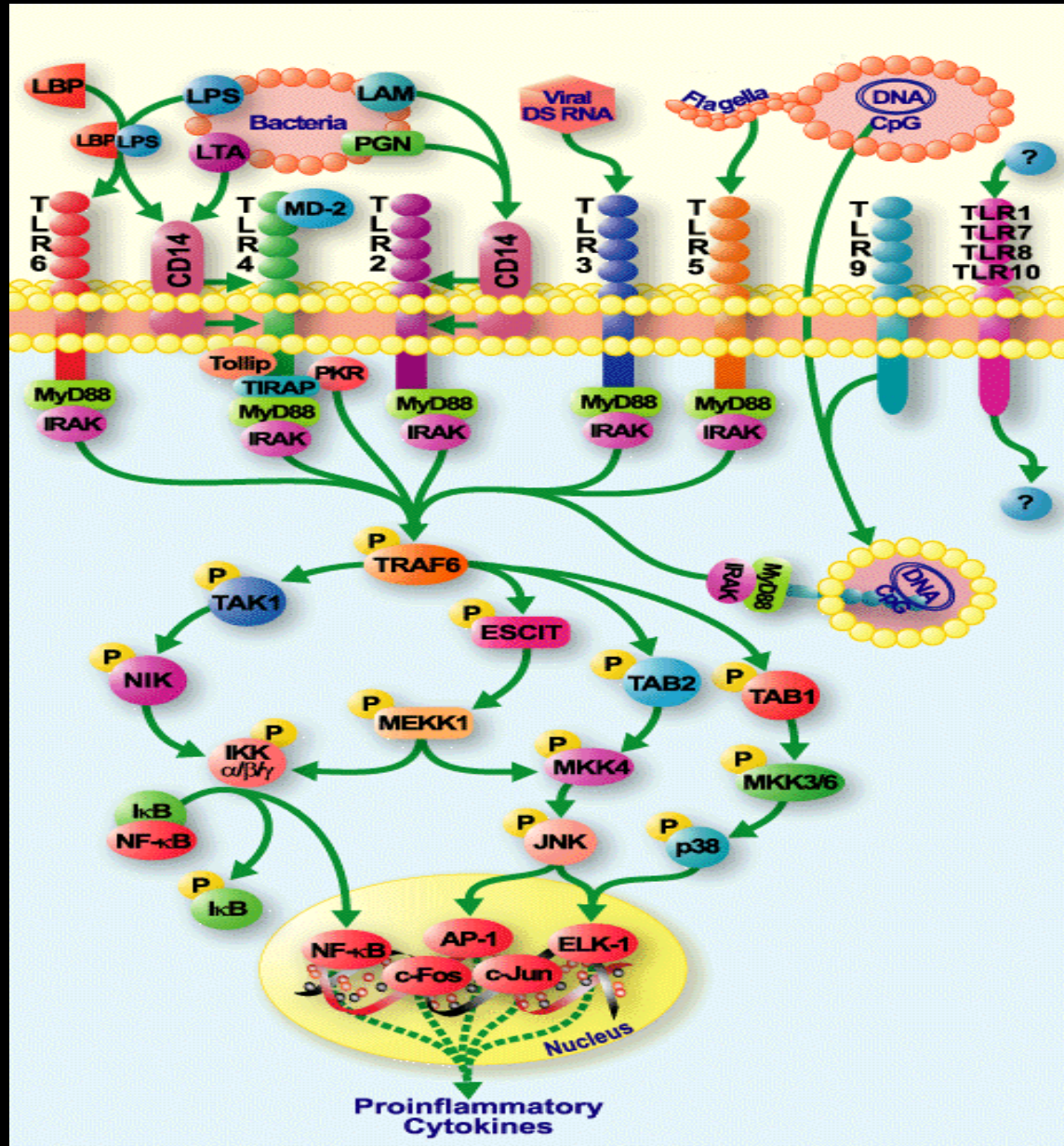
- 10 Receptors (TLR1 – TLR10)
- Specific pathogen detection
- Mediate the innate immune response
- Induce the inflammatory reaction (NF- $\kappa$ B)
- Induce the adaptative immune response
- Resolution of the inflammation (apoptosis)



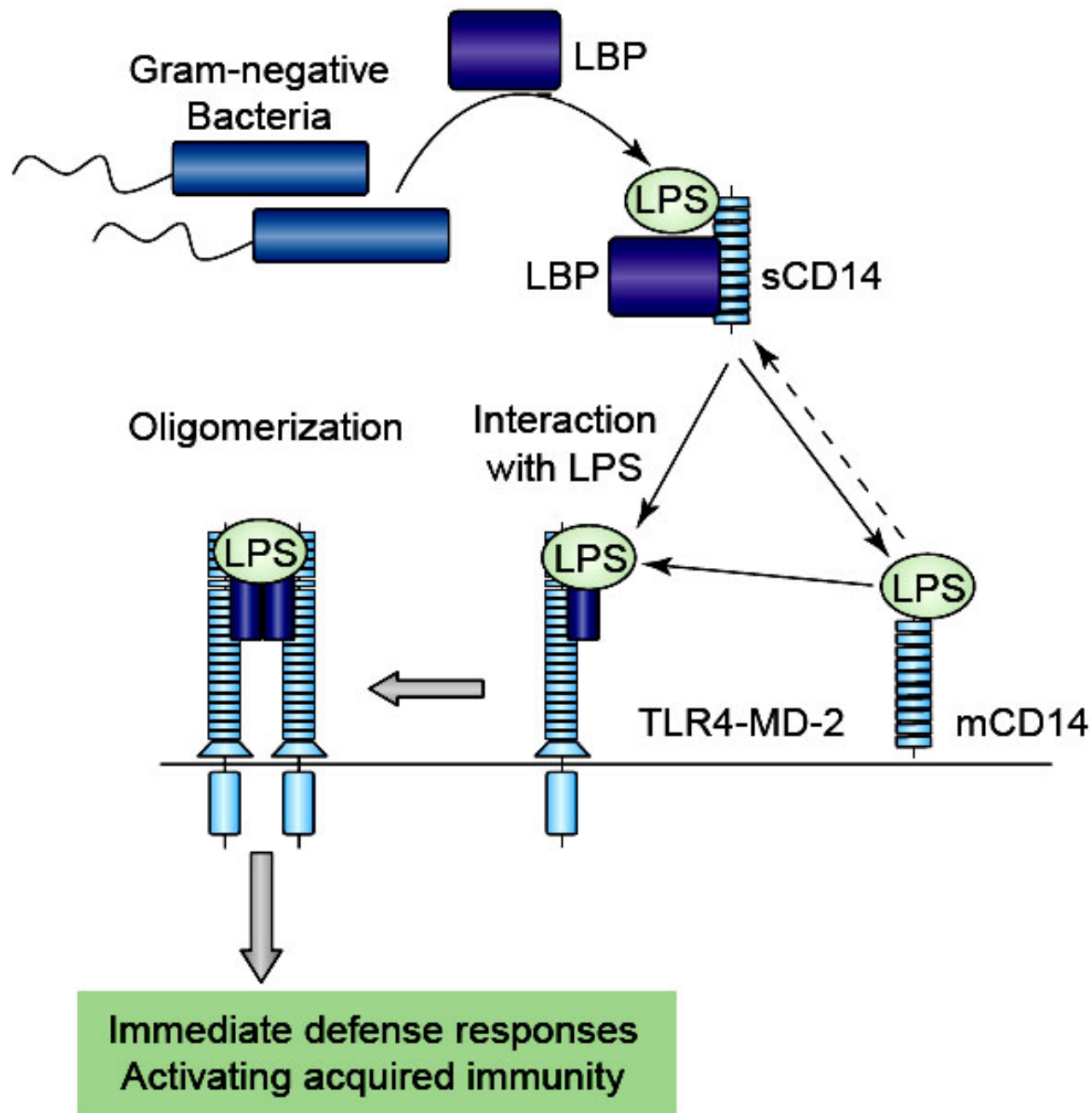
# Polymorphisms of the CD14 receptor



# CD14 receptor and TLRs

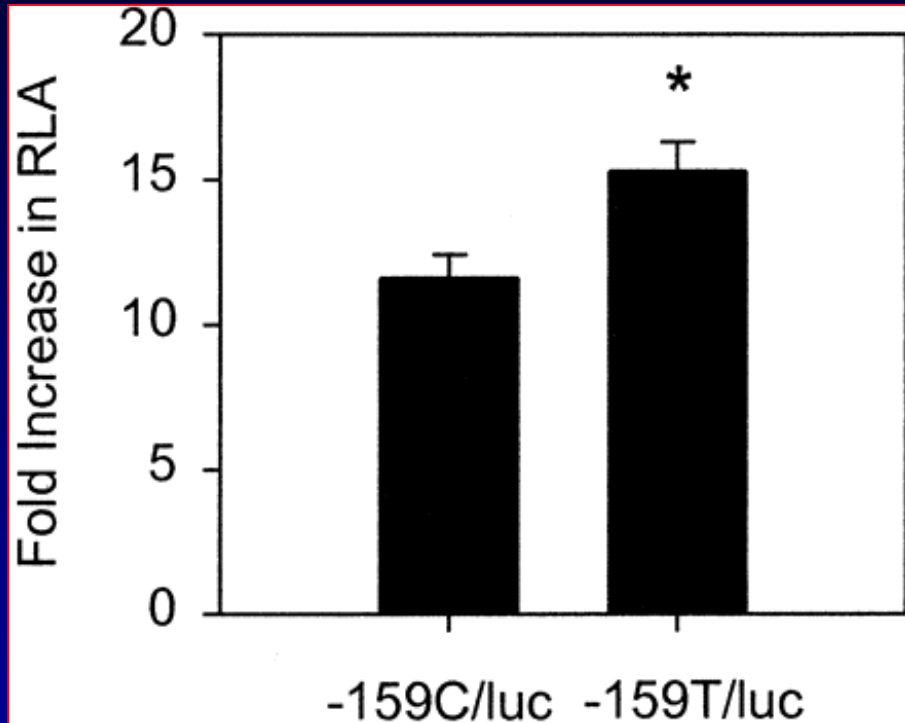




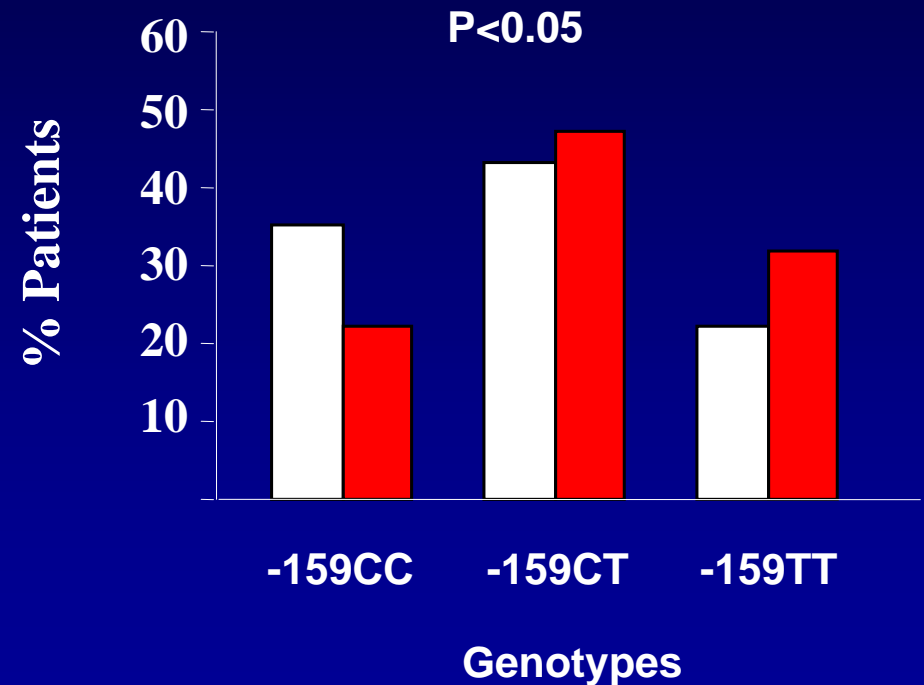


# CD14/159T and Susceptibility to Septic Shock

CD14/159T increases sCD14 and mCD14 levels



Control  
Septic Shock



# CD14/159T and Mortality to Septic Shock

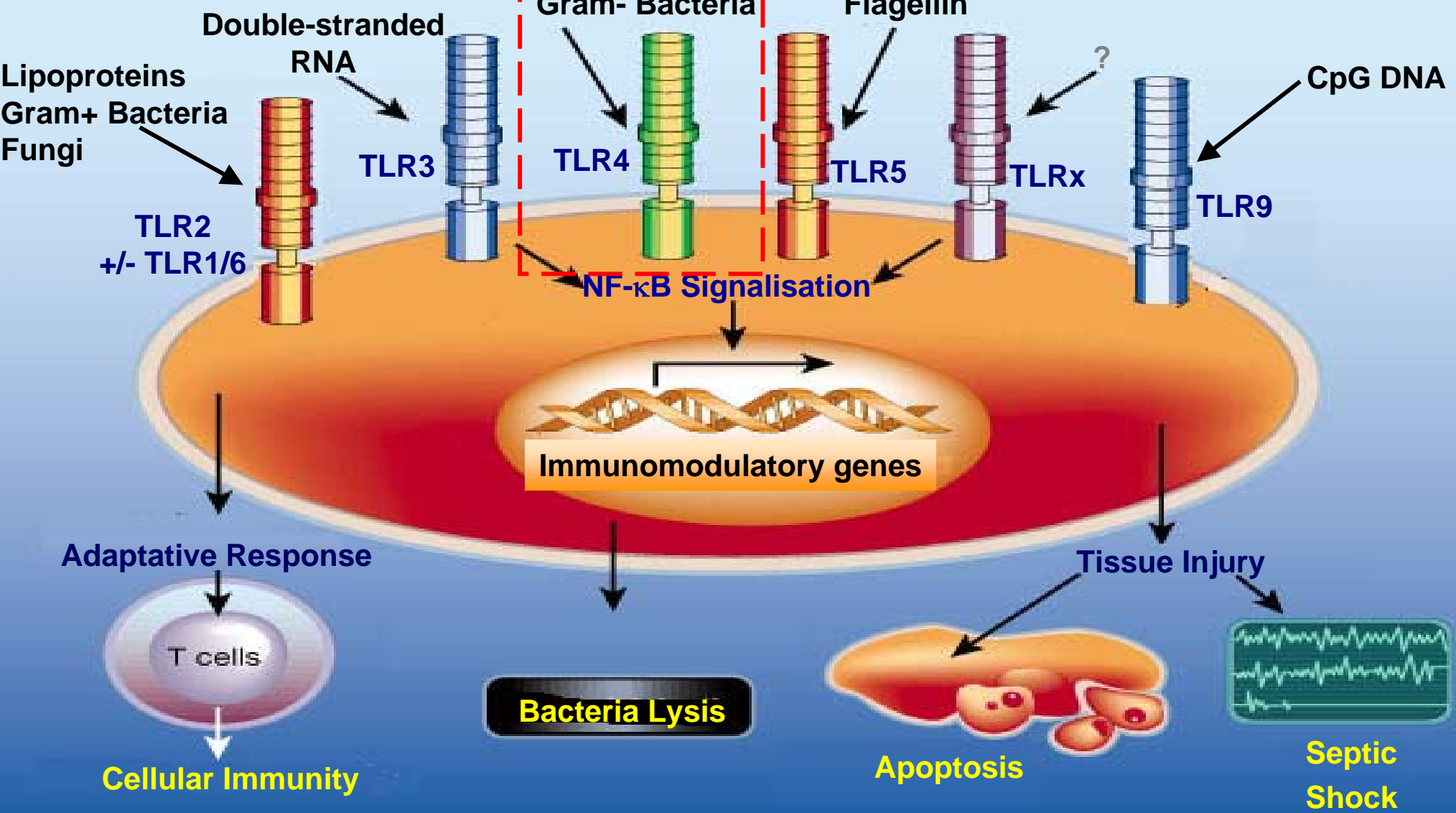
Characteristics	C/C N=19	C/T N=43	T/T N=28	p
Age (mean±SD)	53±15	59±13	59±17	.18
SAPSII (mean±SD)	53±18	56±21	60±19	.21
OSF (mean±SD)	3±1.2	2.8±0.9	3.1±1	.42

# CD14/159T and Mortality to Septic Shock

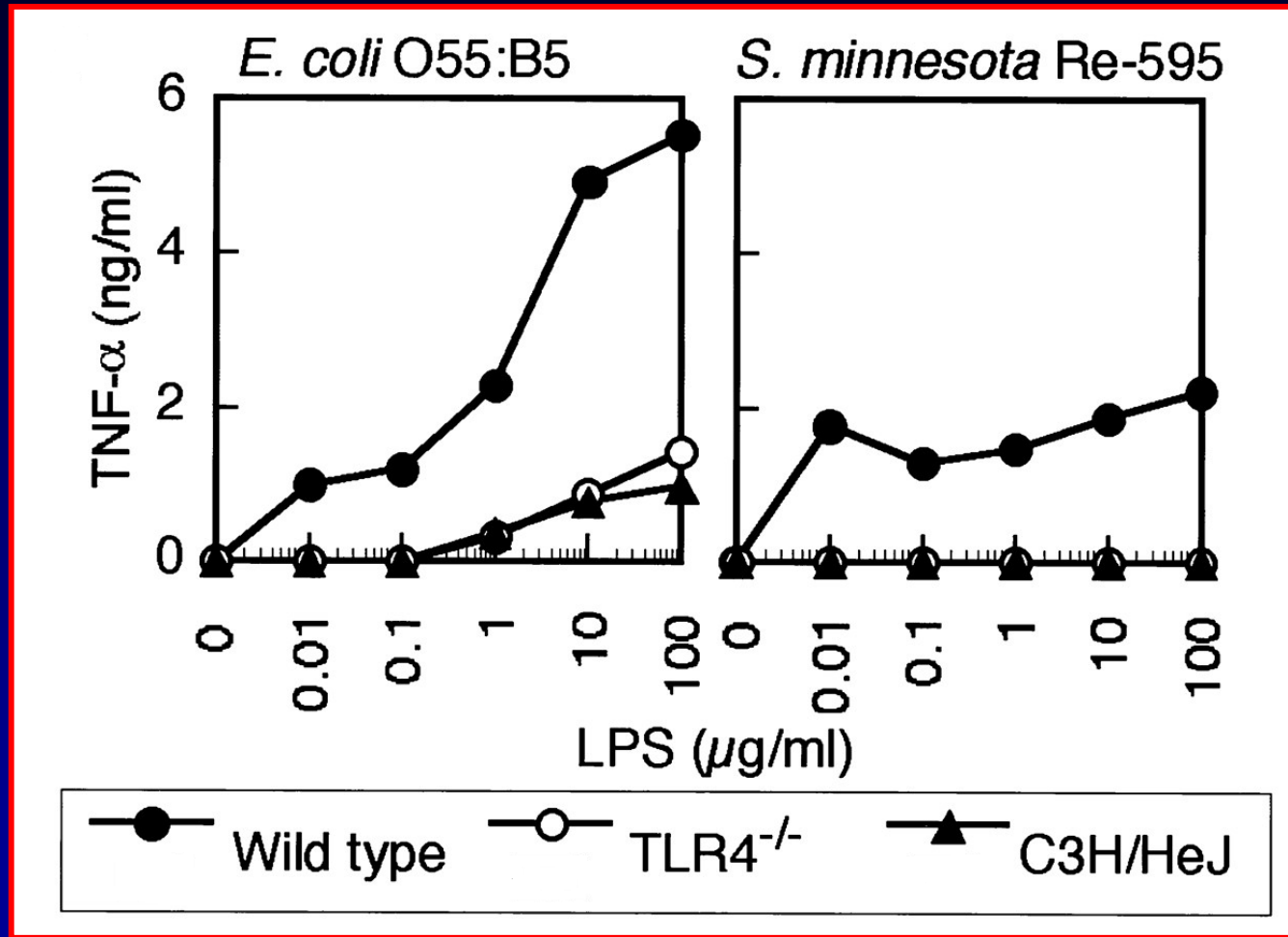
Characteristics	C/C N=19	C/T N=43	T/T N=28	p
Age (mean±SD)	53±15	59±13	59±17	.18
SAPSII (mean±SD)	53±18	56±21	60±19	.21
OSF (mean±SD)	3±1.2	2.8±0.9	3.1±1	.42
<b>Mortality (%)</b>	<b>26.3</b>	<b>58.1</b>	<b>71.4</b>	<b>&lt;.0001</b>

CD14/159TT RR= 5.1; 95%CI [3.2-7,9]

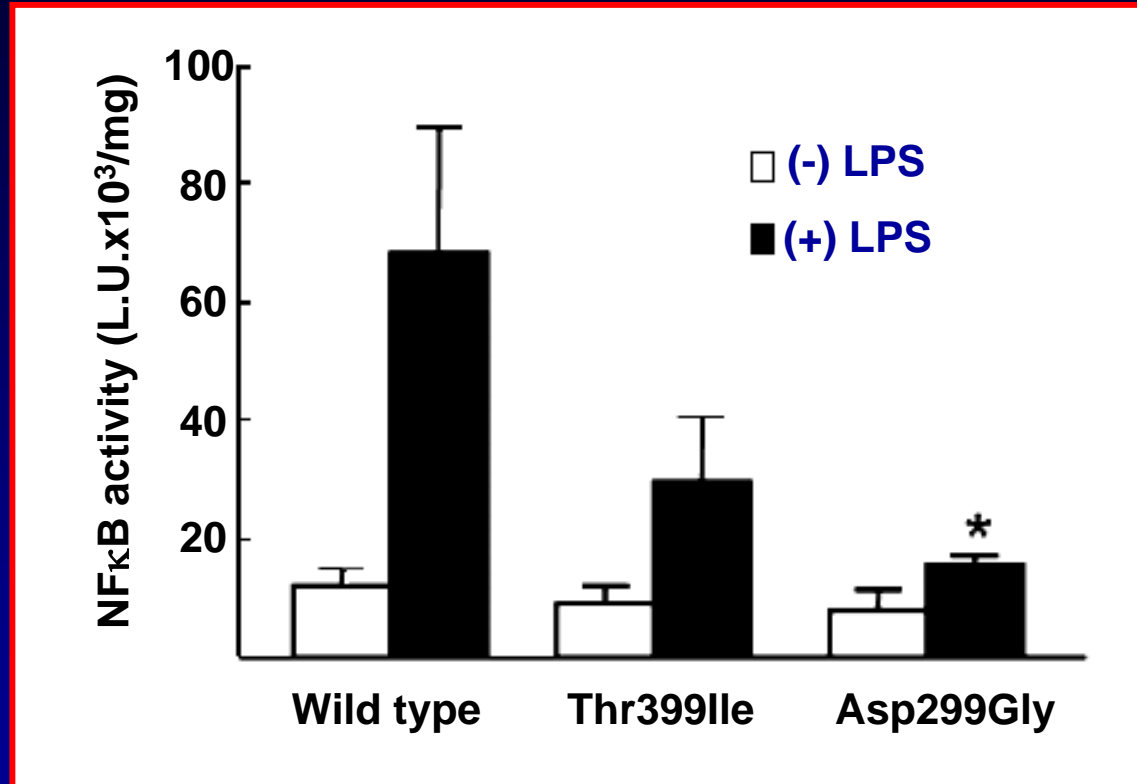
# Monocyte or Dendritic cell



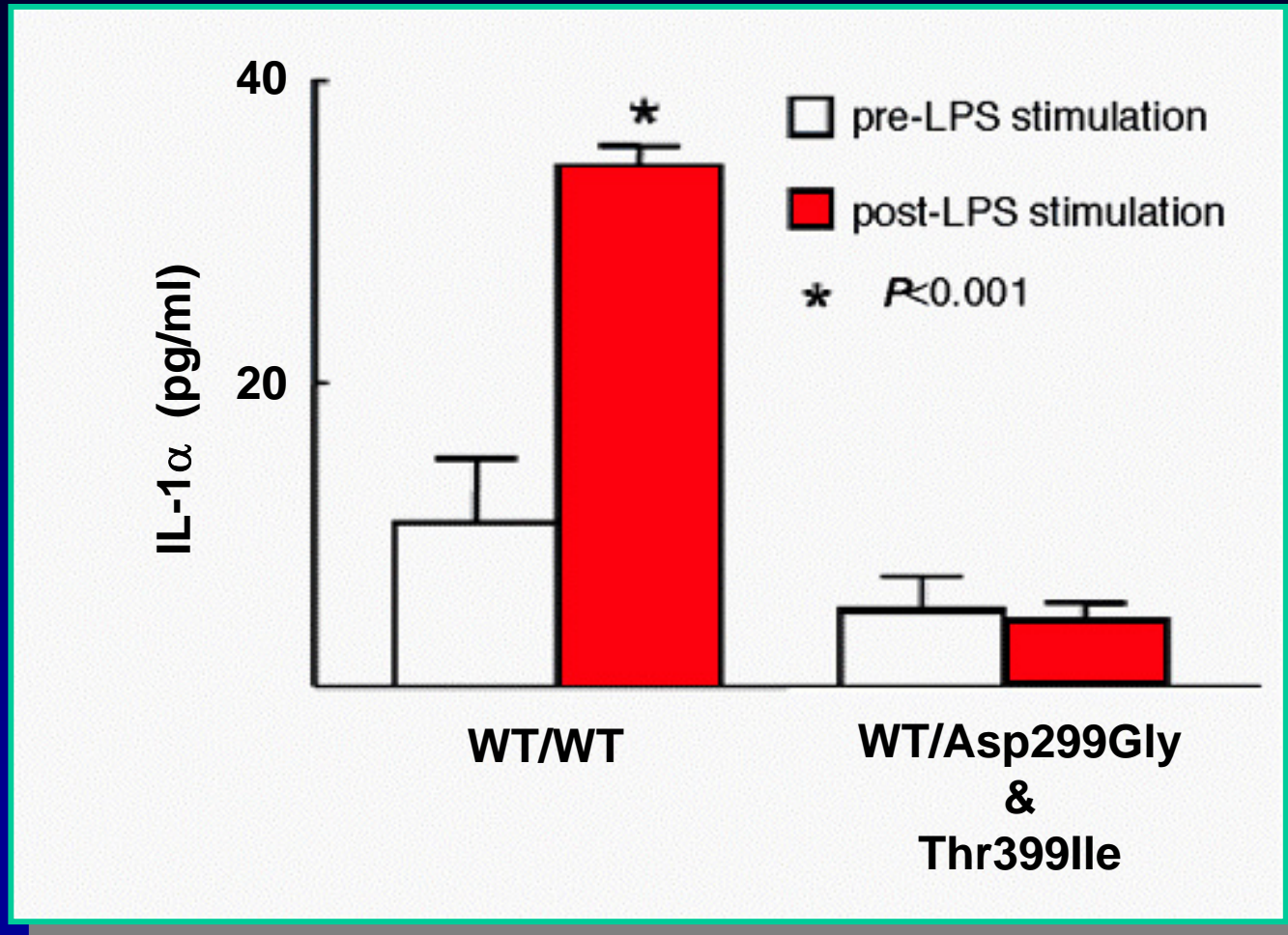
# Impaired LPS responsiveness in TLR4<sup>-/-</sup> macrophages



# NF- $\kappa$ B Activity in Transfected THP-1 cells



# TLR4 mutation and LPS responsiveness

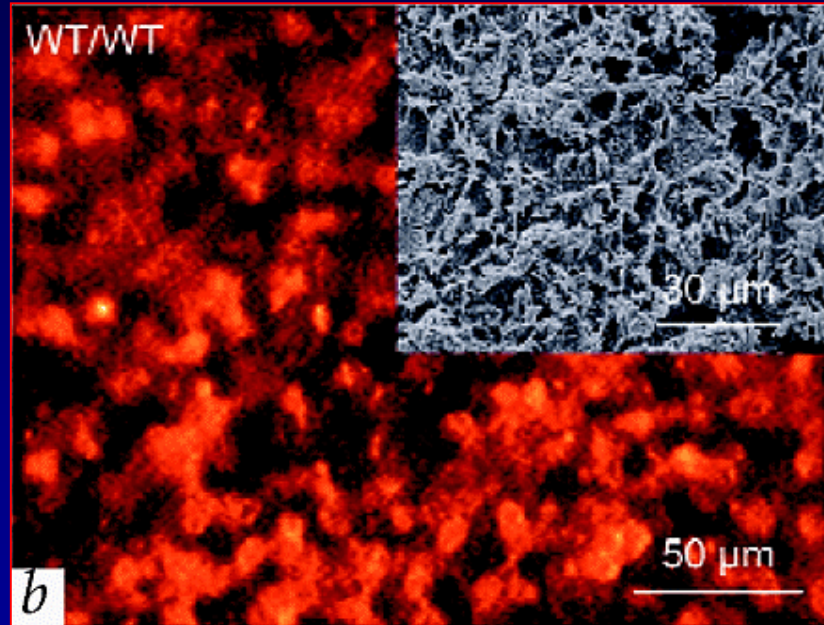




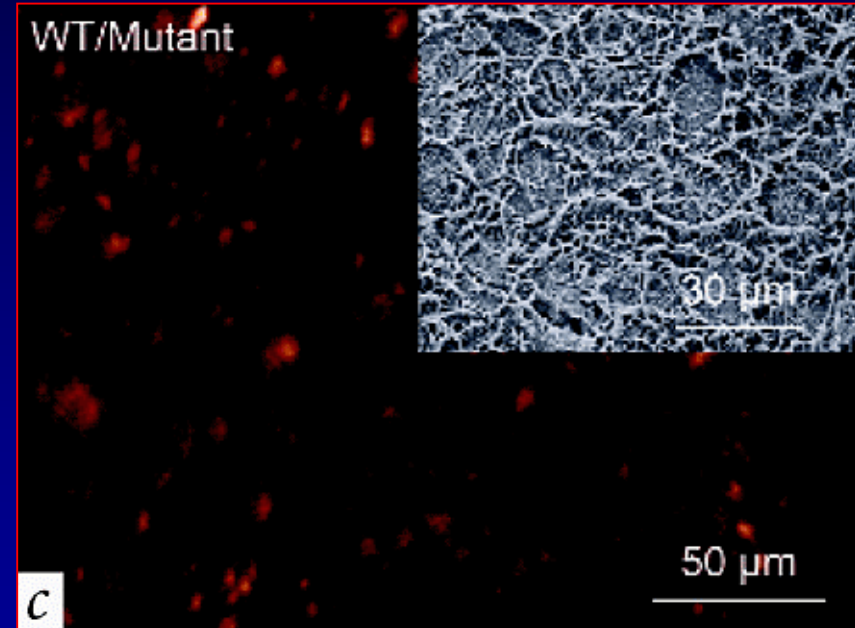
# TLR4 Mutant Expression

## Human Airway Epithelia

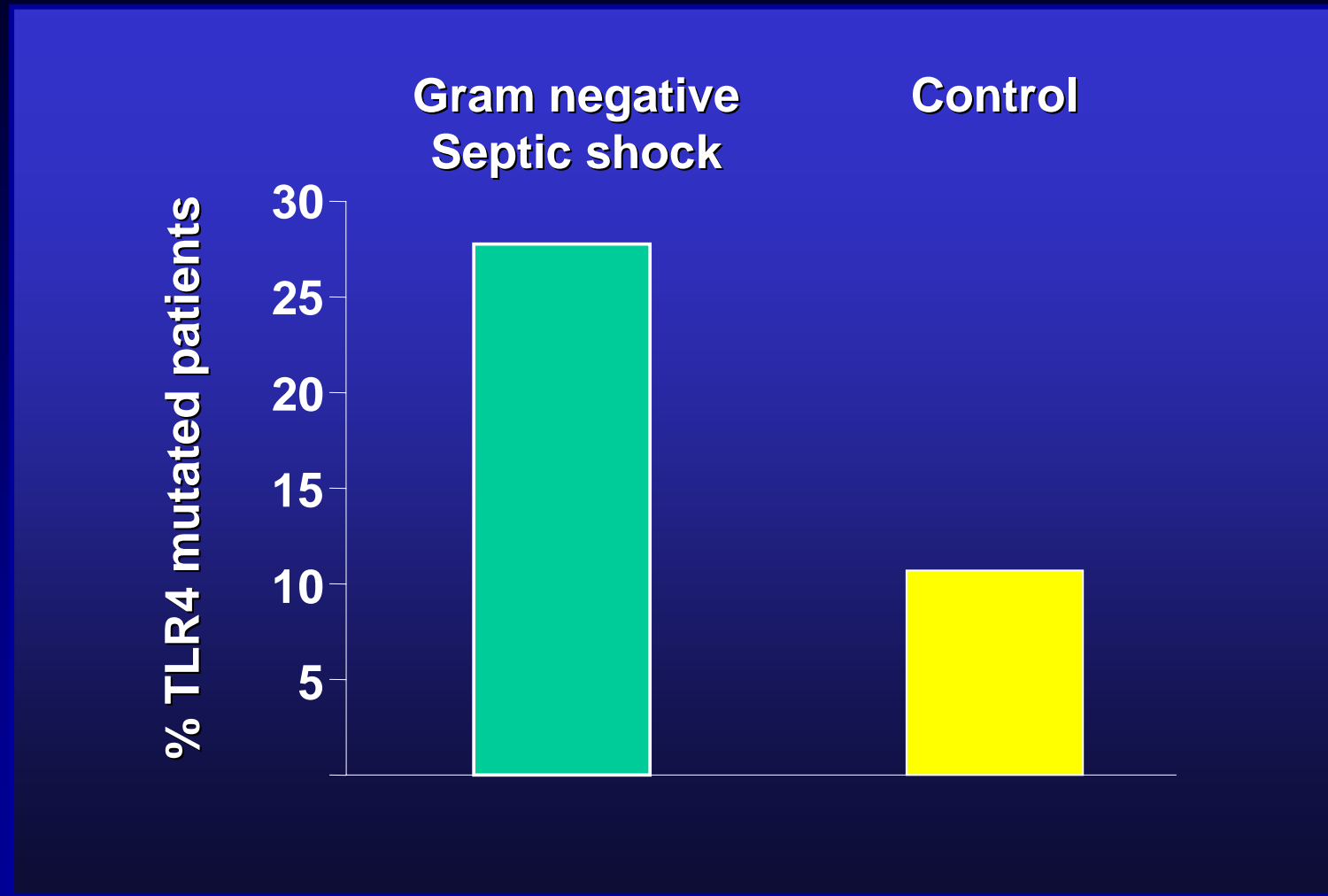
TLR4 WT



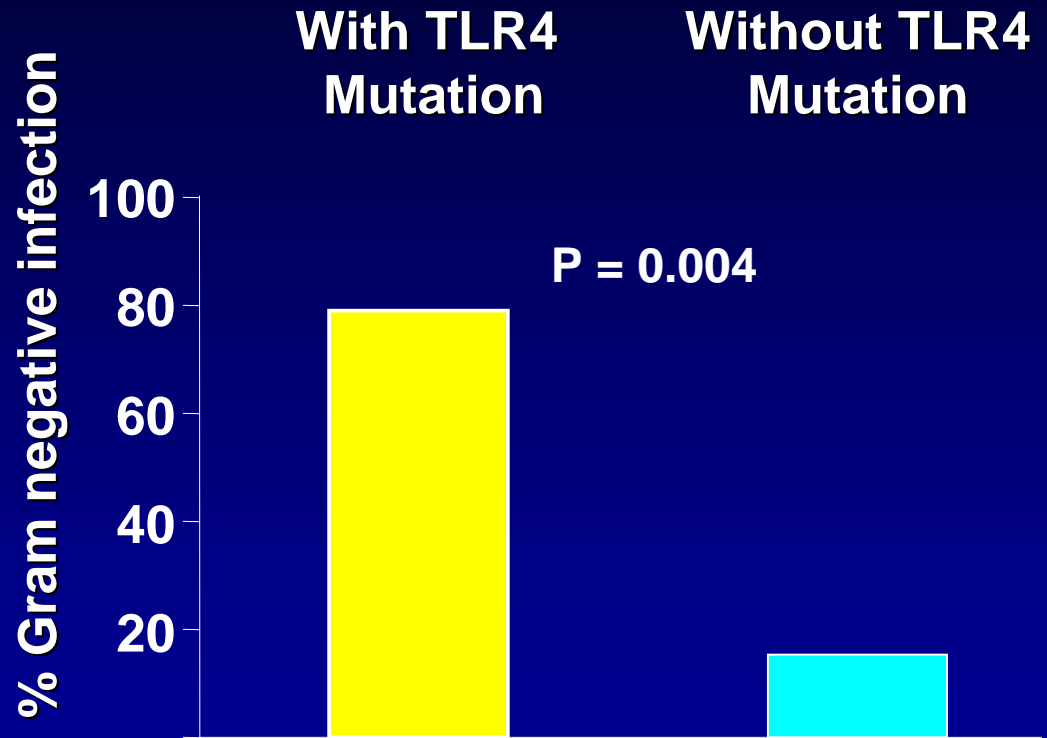
TLR4 WT/299 Gly



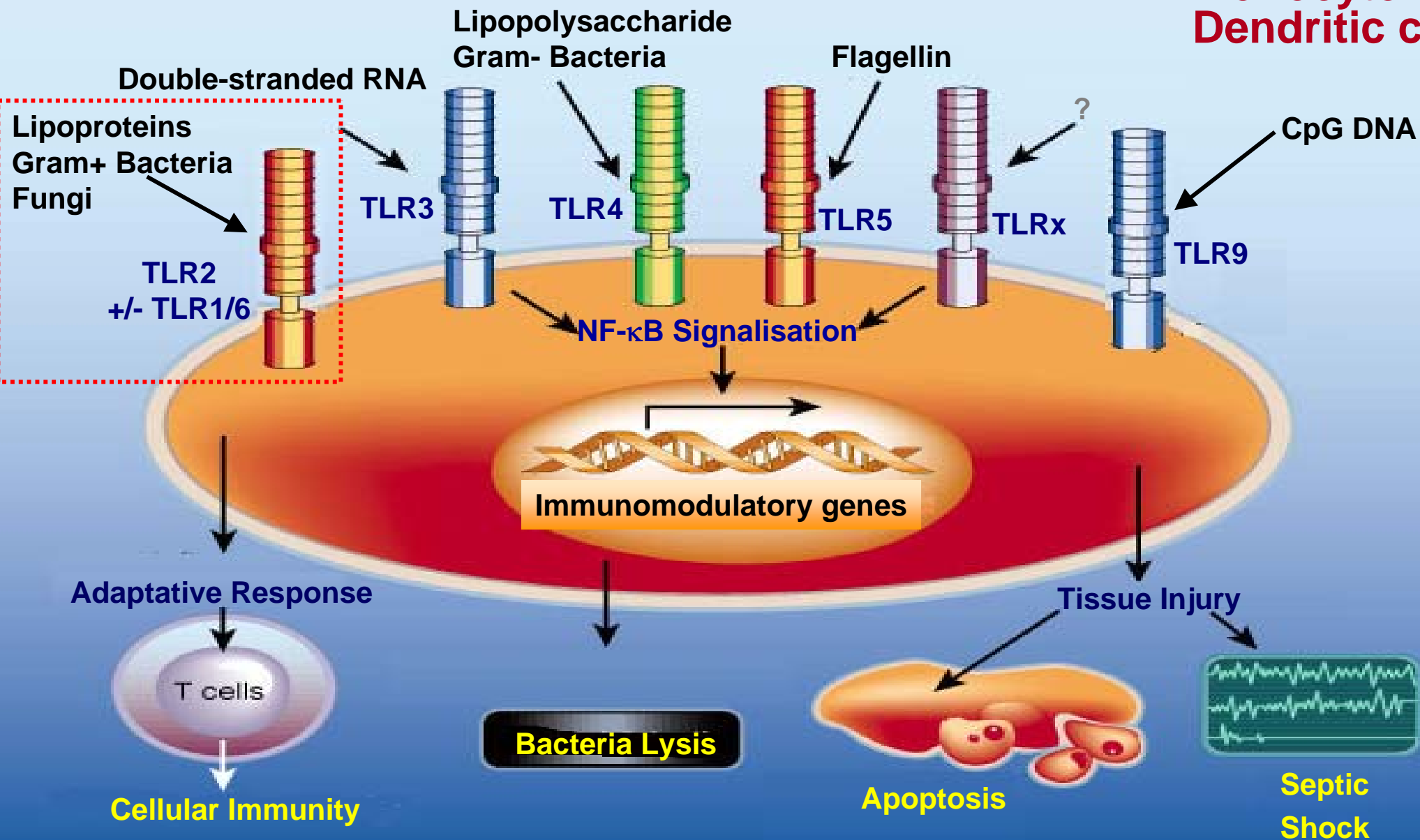
# TLR4 Polymorphisms and Septic Shock



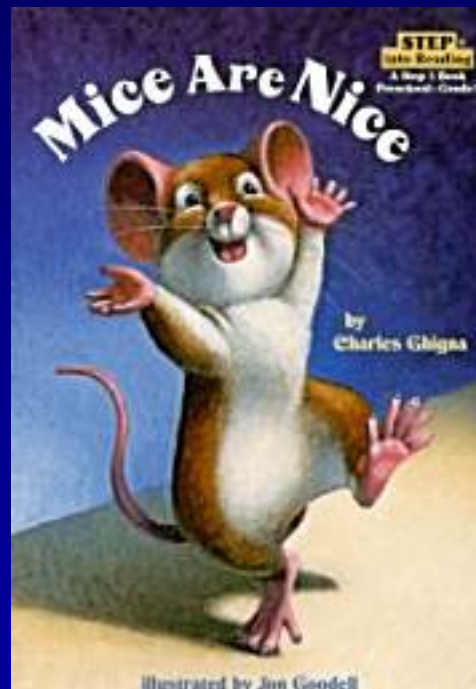
# TLR4 Variants and Predisposition to Gram Negative Sepsis



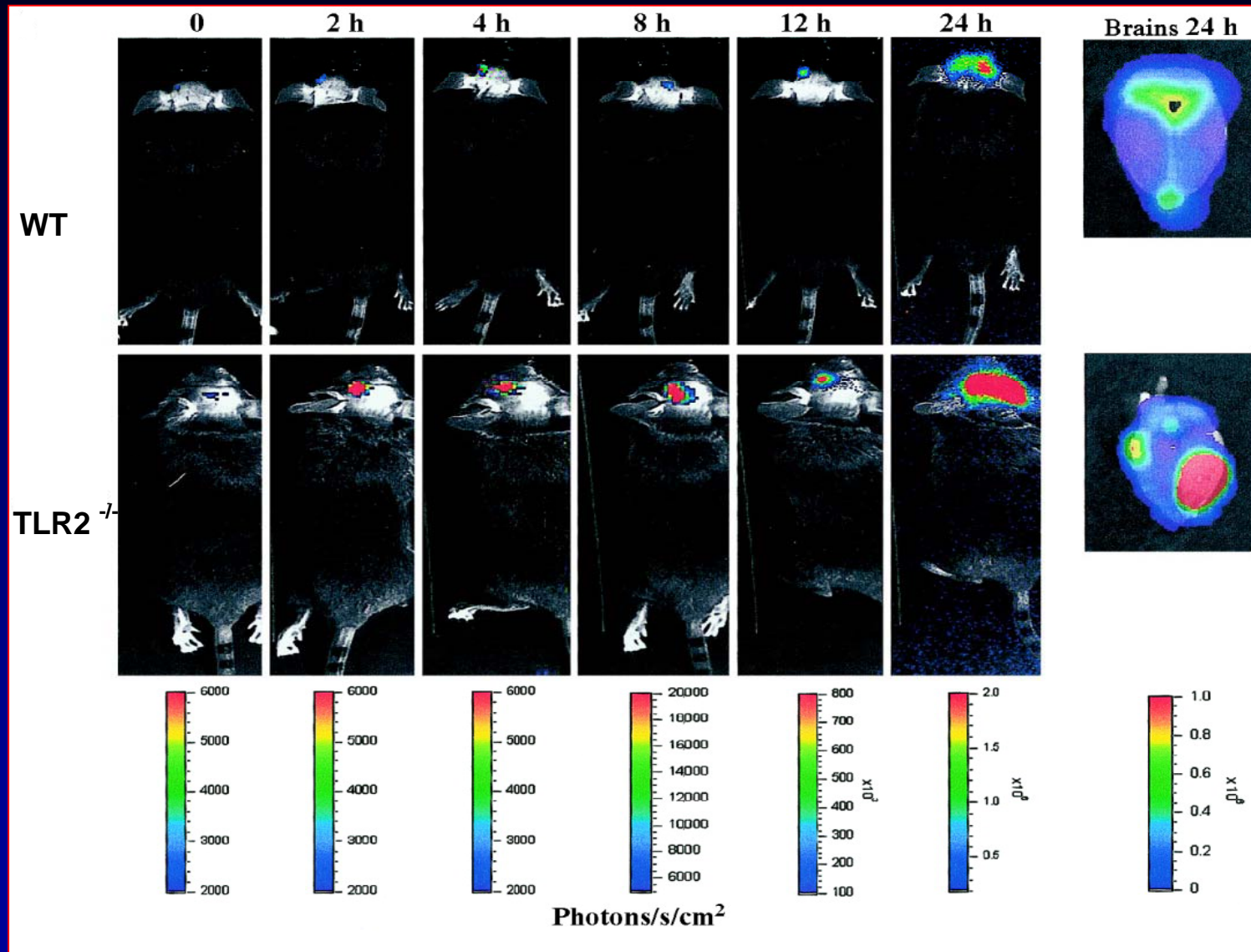
# Monocyte or Dendritic cell



# TLR2-Knockout Mice

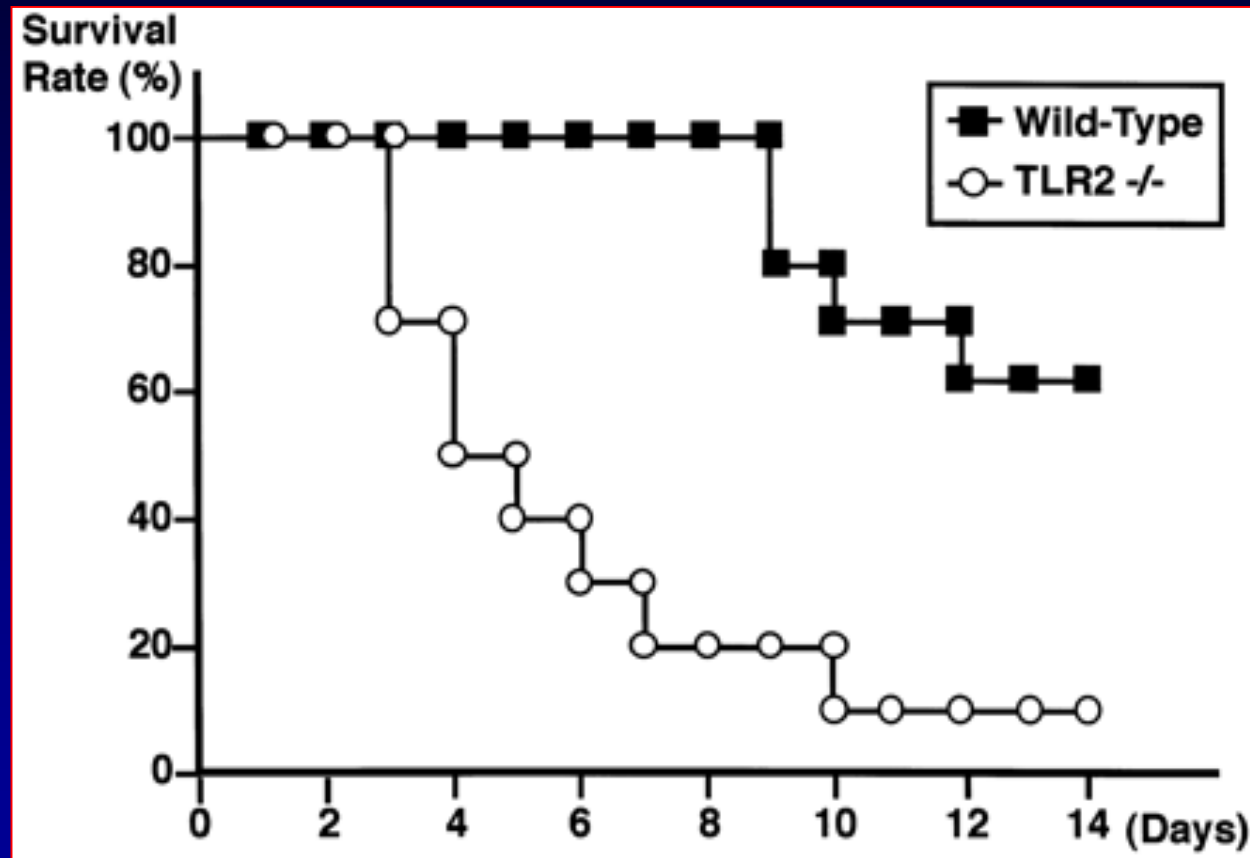


# TLR2 and *Streptococcus pneumoniae* meningitis



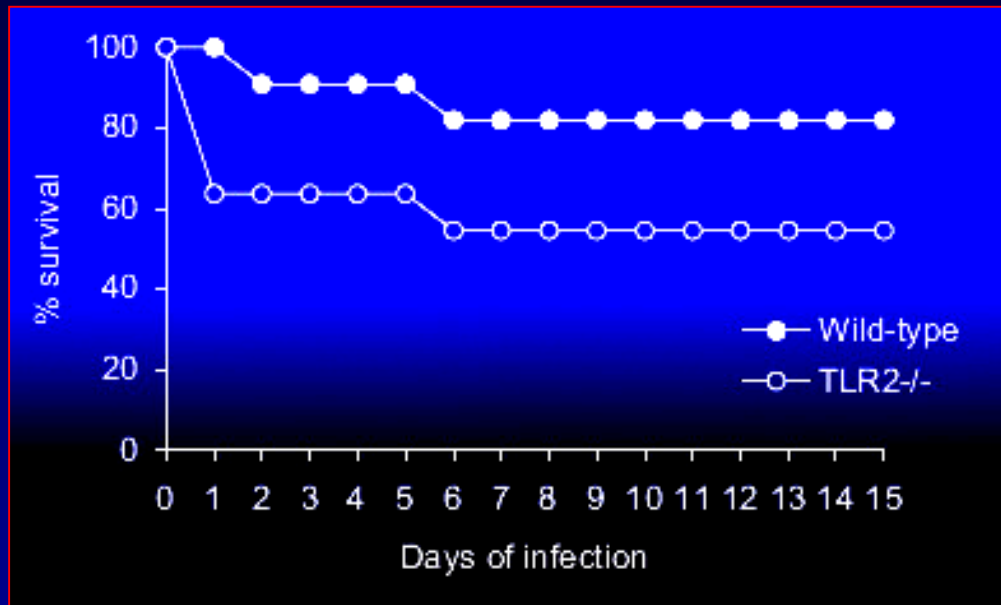
# TLR2-deficient mice and Gram positive infections

## IV challenge of lived *Staphylococcus aureus*

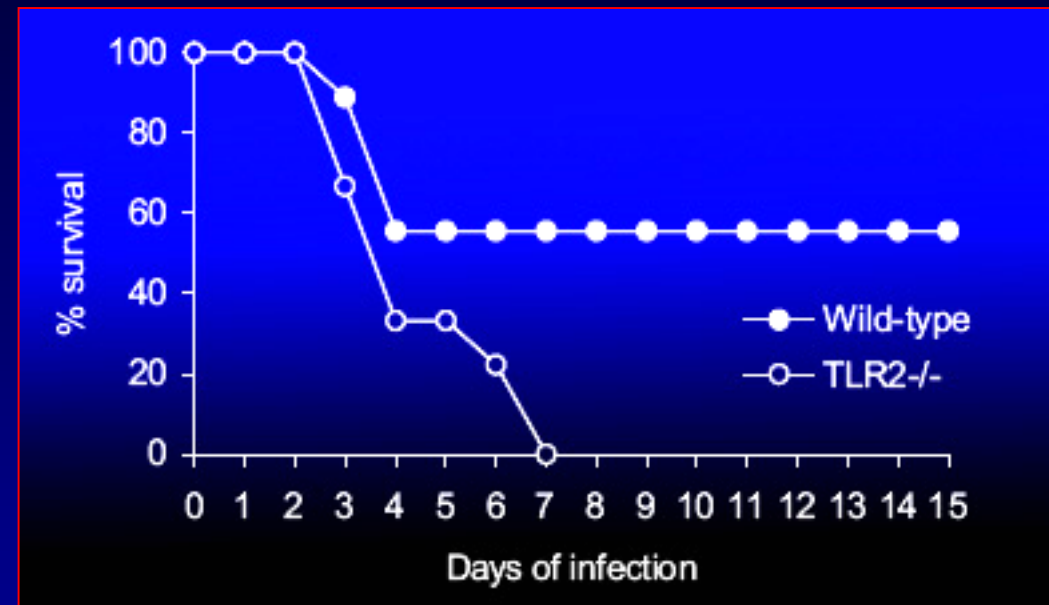


# TLR2-deficient mice and Candida Infections

*Candida albicans* IP  $50 \times 10^6$



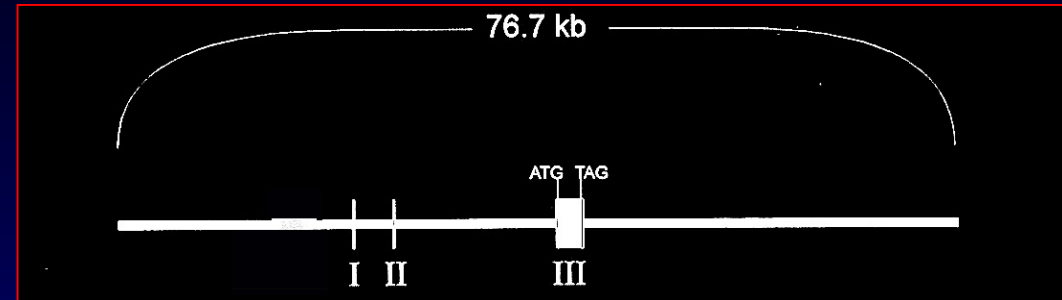
*Candida albicans* IV  $10^6$





# TLR2 Polymorphisms in Humans

*Tlr2* location: Chromosome 4q32



<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>

<http://innateimmunity.net>

**88 SNPs**

- **5'UTR: 26 SNPs**
- **3'UTR: 17 SNPs**
- **Intron: 29 SNPs**
- **Exon: 16 SNPs**

## Synonymous

Position rs number  
35 5743697

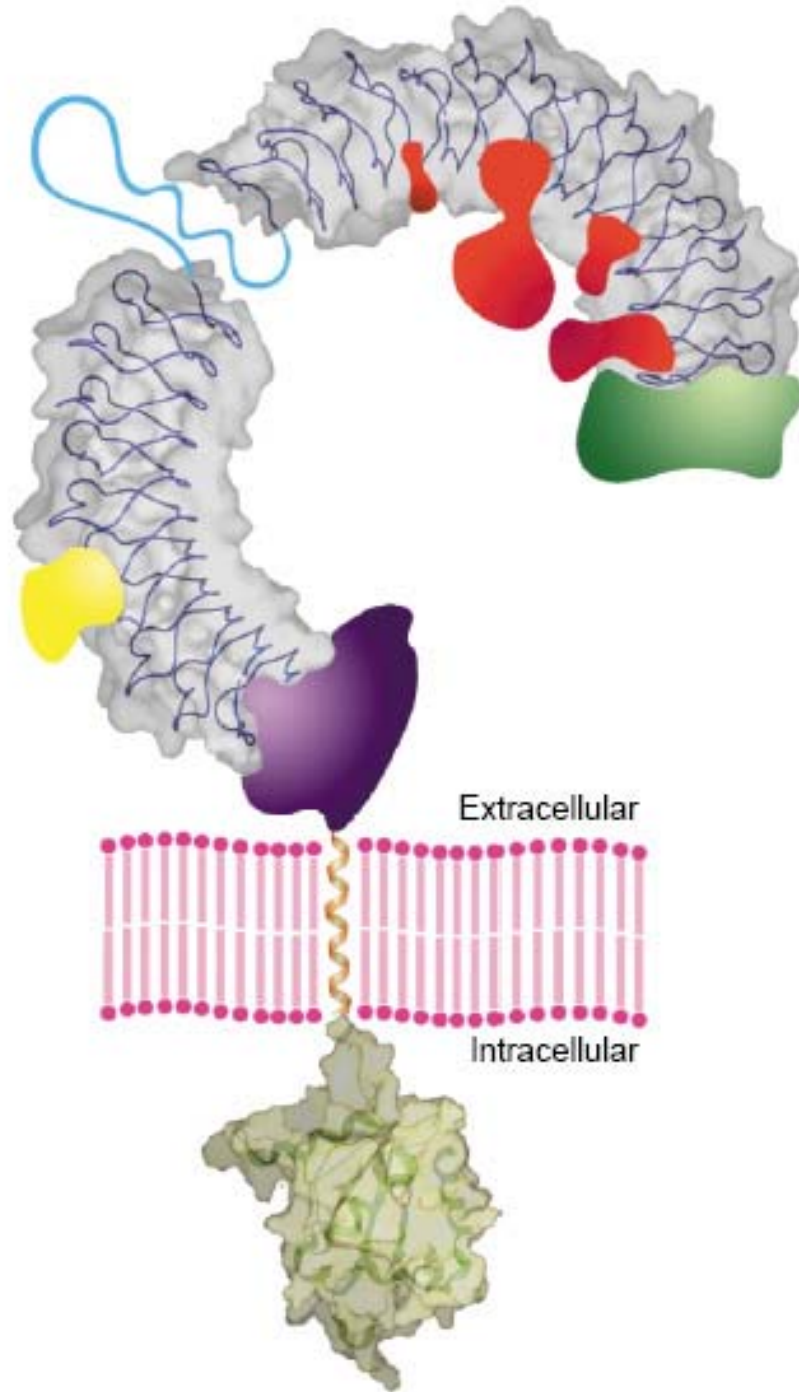
199 3804099  
213 5743698

450 3804100

541 5743700  
542 5743701

707 IIPGA-TLR2-31410

781 5743709



## Non-synonymous

Position rs number

411(T/I) 5743699

556 (I/T) 5743702

579 (R/H) 5743703

631 (P/H) 5743704

677 (R/W) 5743706

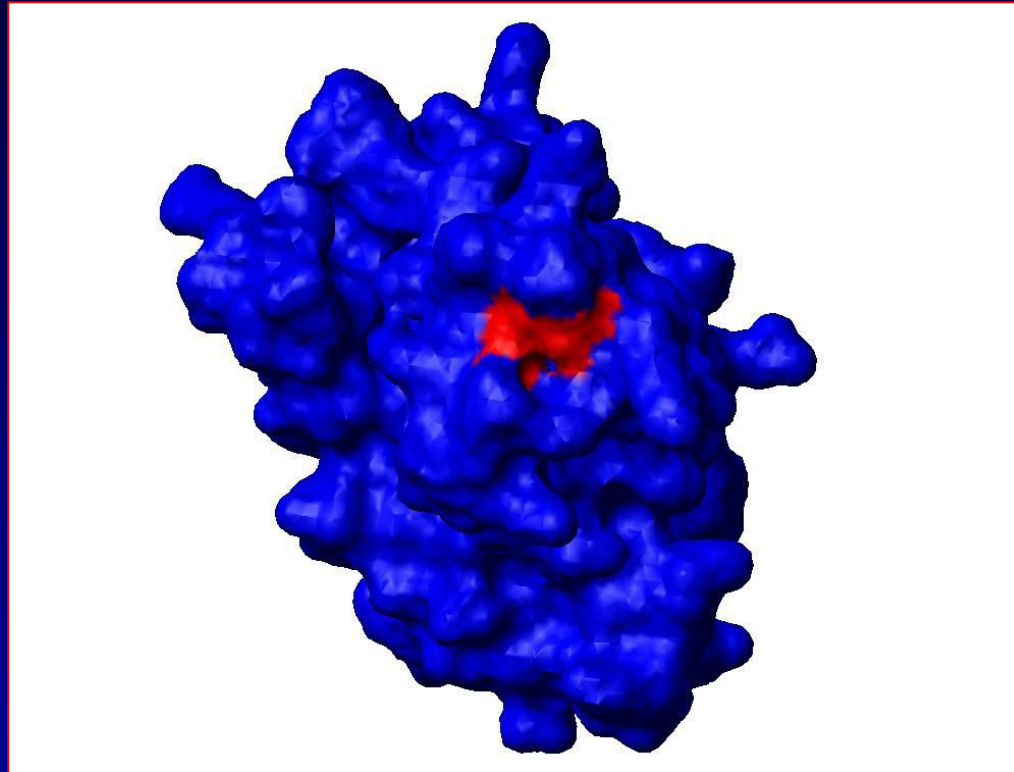
715 (Y/N) 5743707

715 (Y/Amb) 5743707

753 (R/Q) 5743708

# Location of R753 in the TIR domain

---



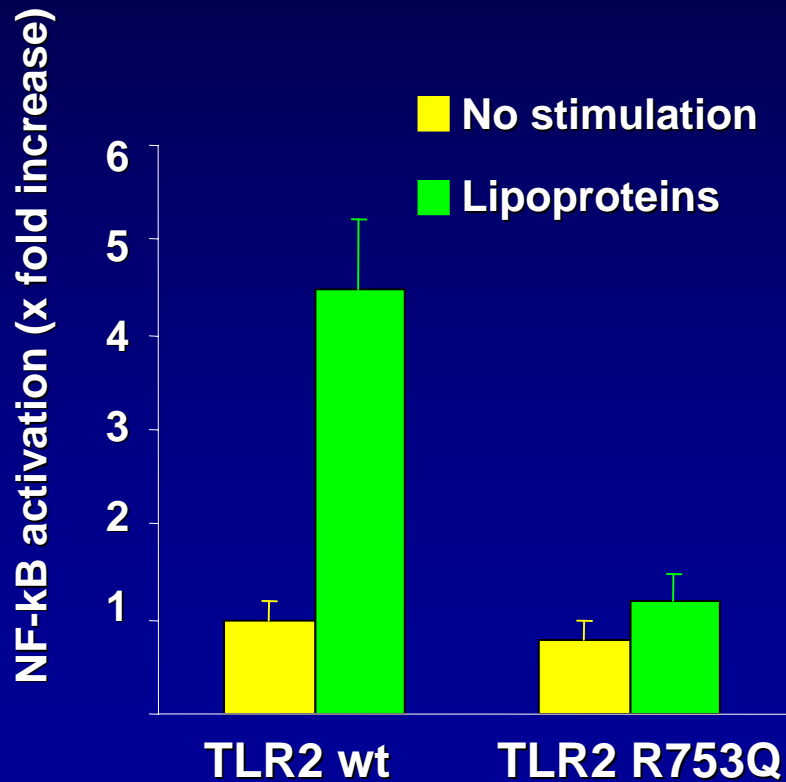
# R753Q Polymorphism in the TLR2 gene

743

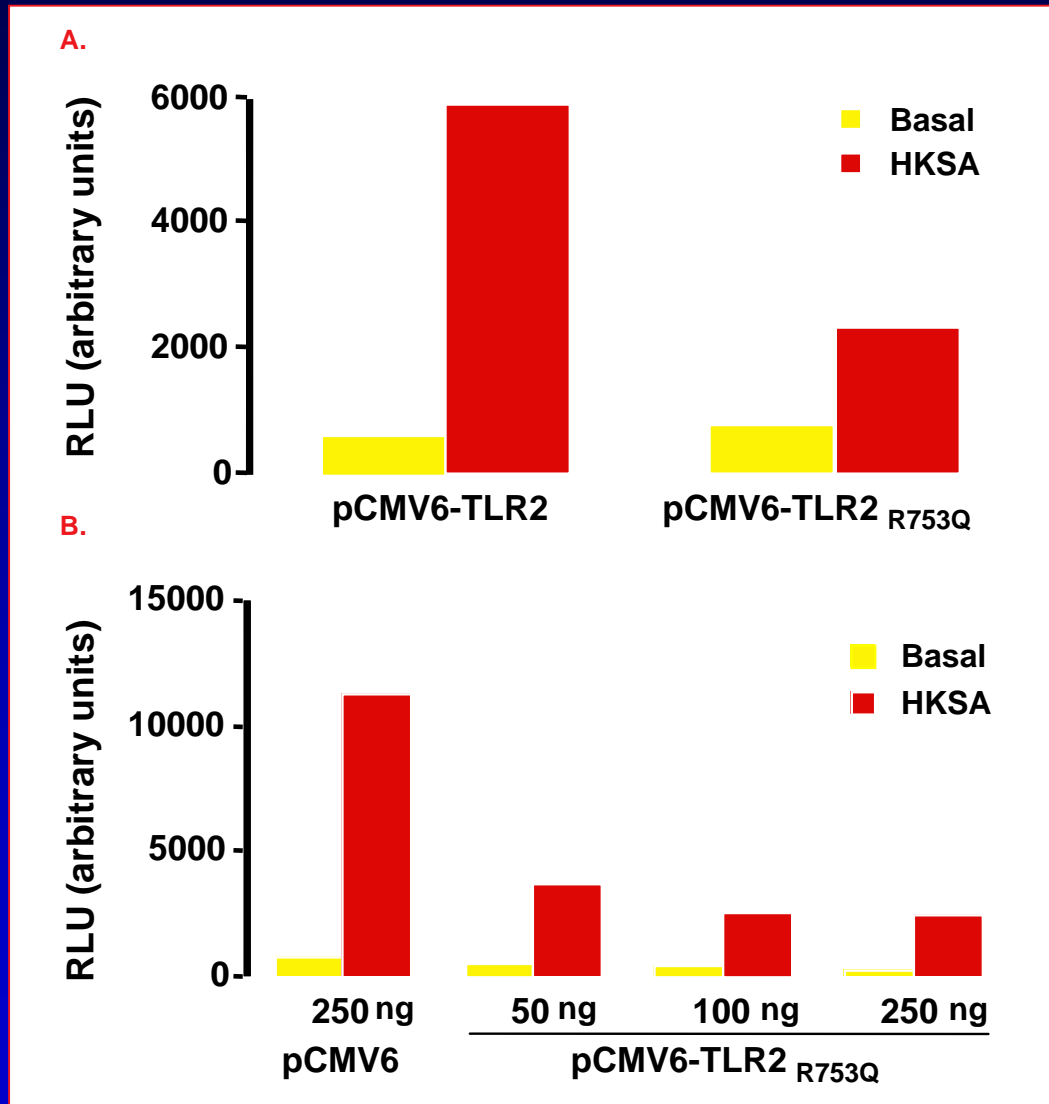
765

Human TLR2 K A I P Q R P C K L **R** K I M N T K T Y L E W P  
Mouse TLR2 K A I P Q R P C K L **R** K I M N T K T Y L E W P

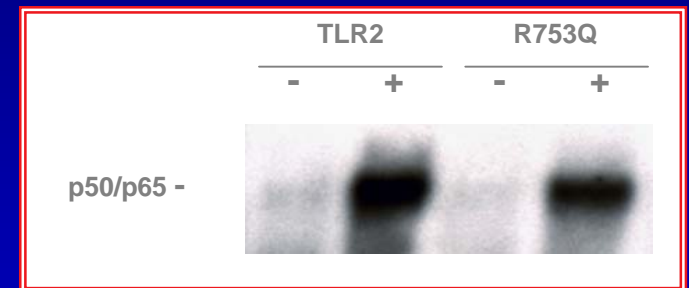
↓  
Q

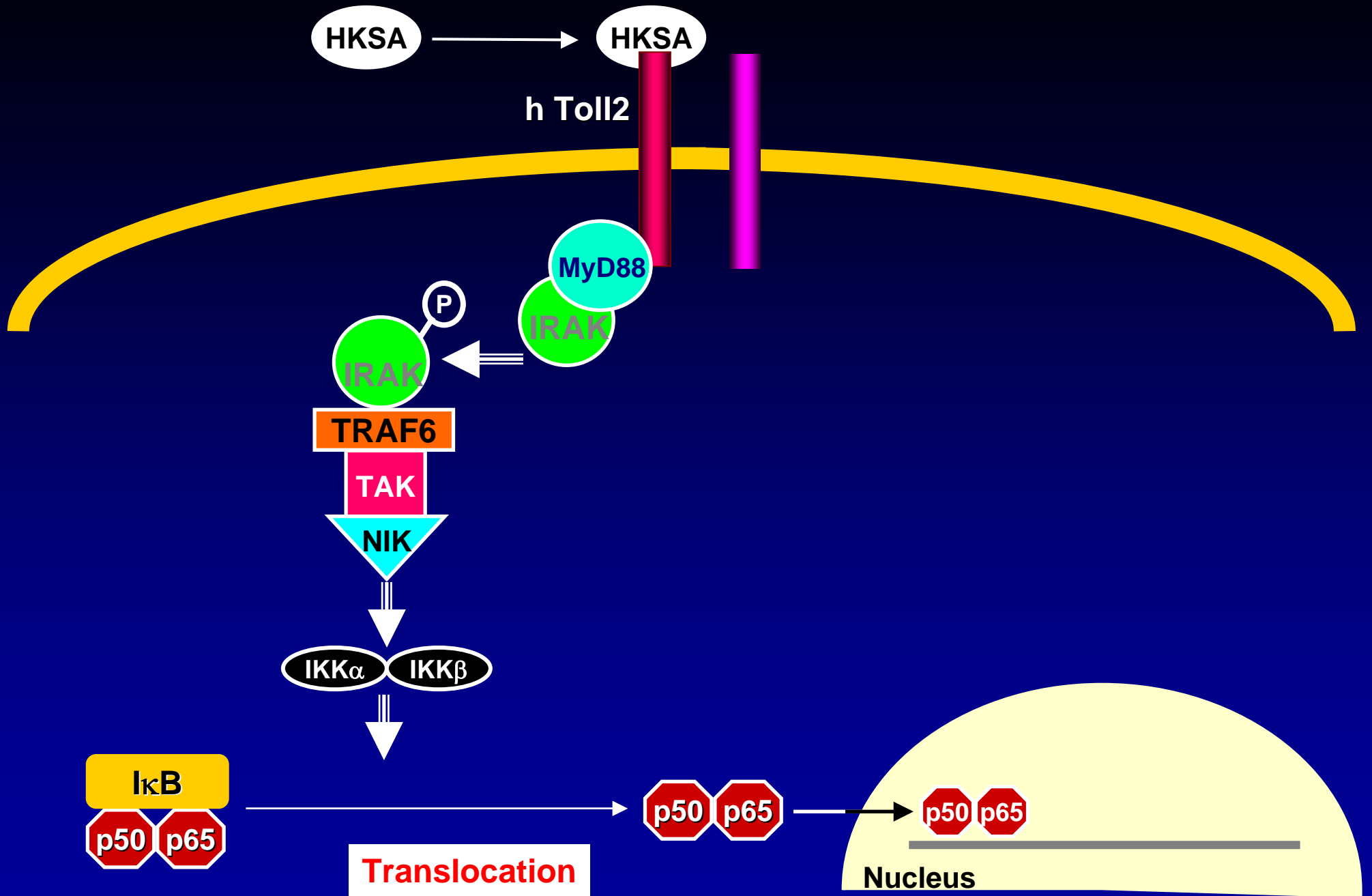


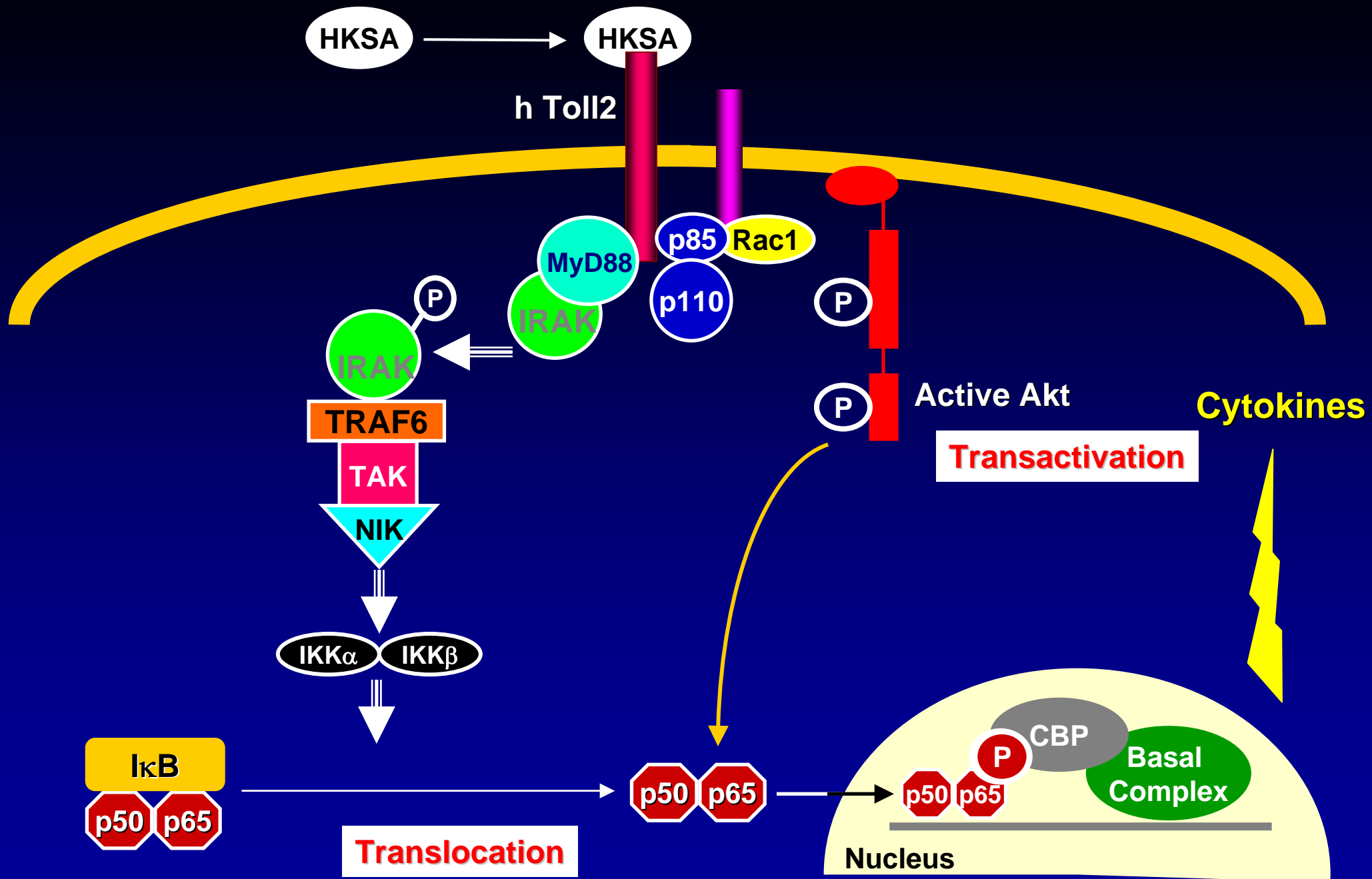
# Dominant Negative Effect of TLR2 R753Q

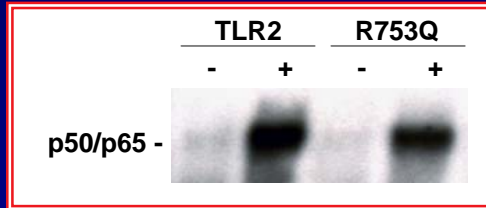
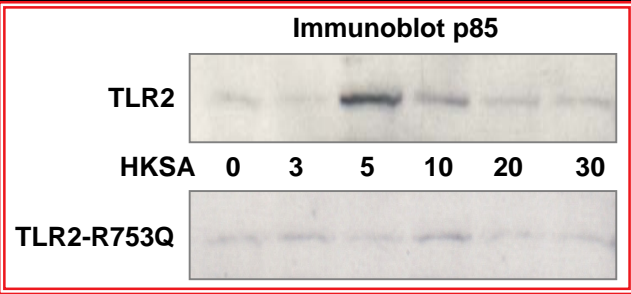
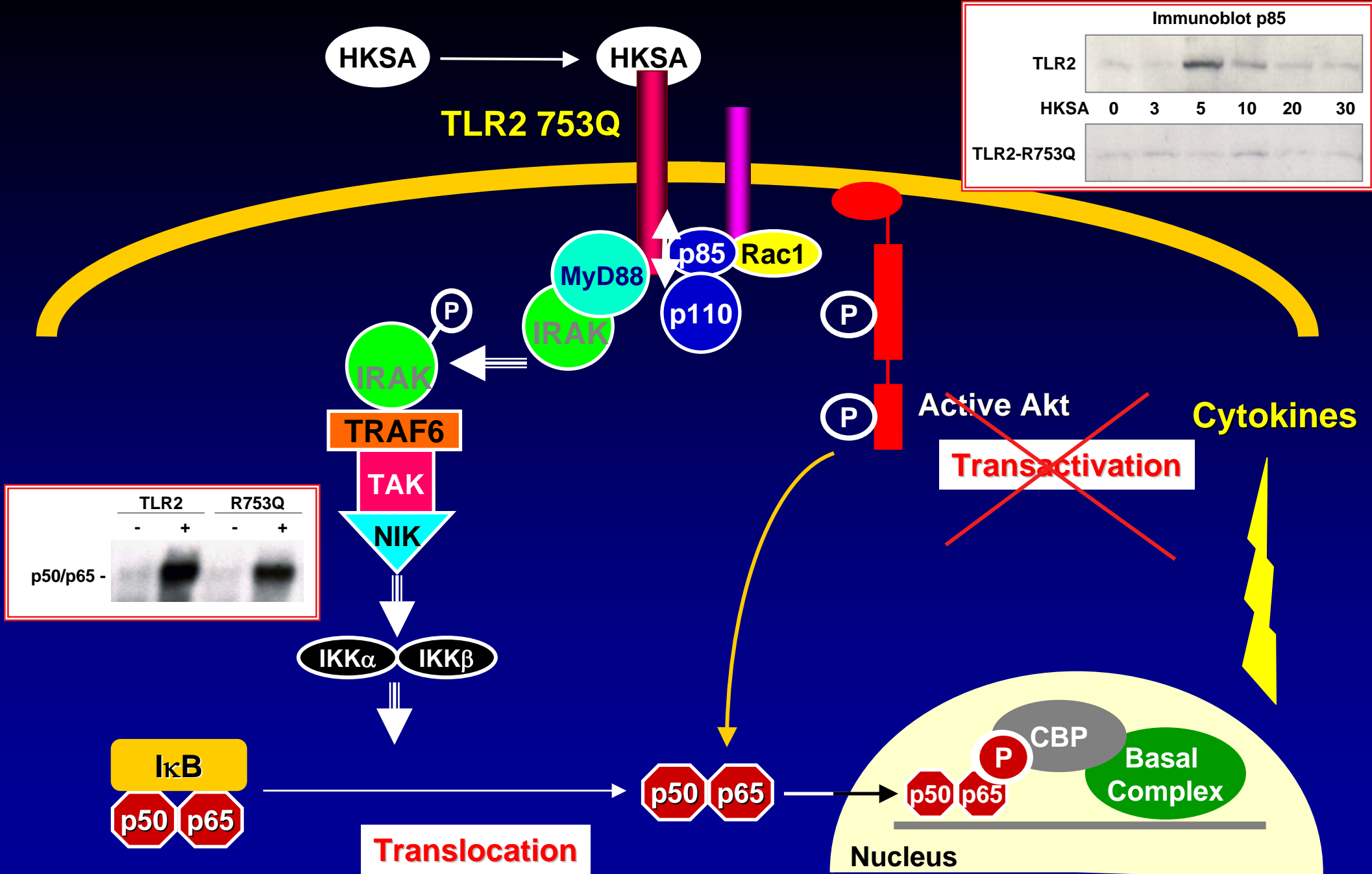


## R753Q and NF- $\kappa$ B translocation













# Imaging HKSA-induced PI-3K activation

**TLR2**

**R753Q**

QuickTime™ et un décompresseur  
Cinepak sont requis pour visualiser  
cette image.

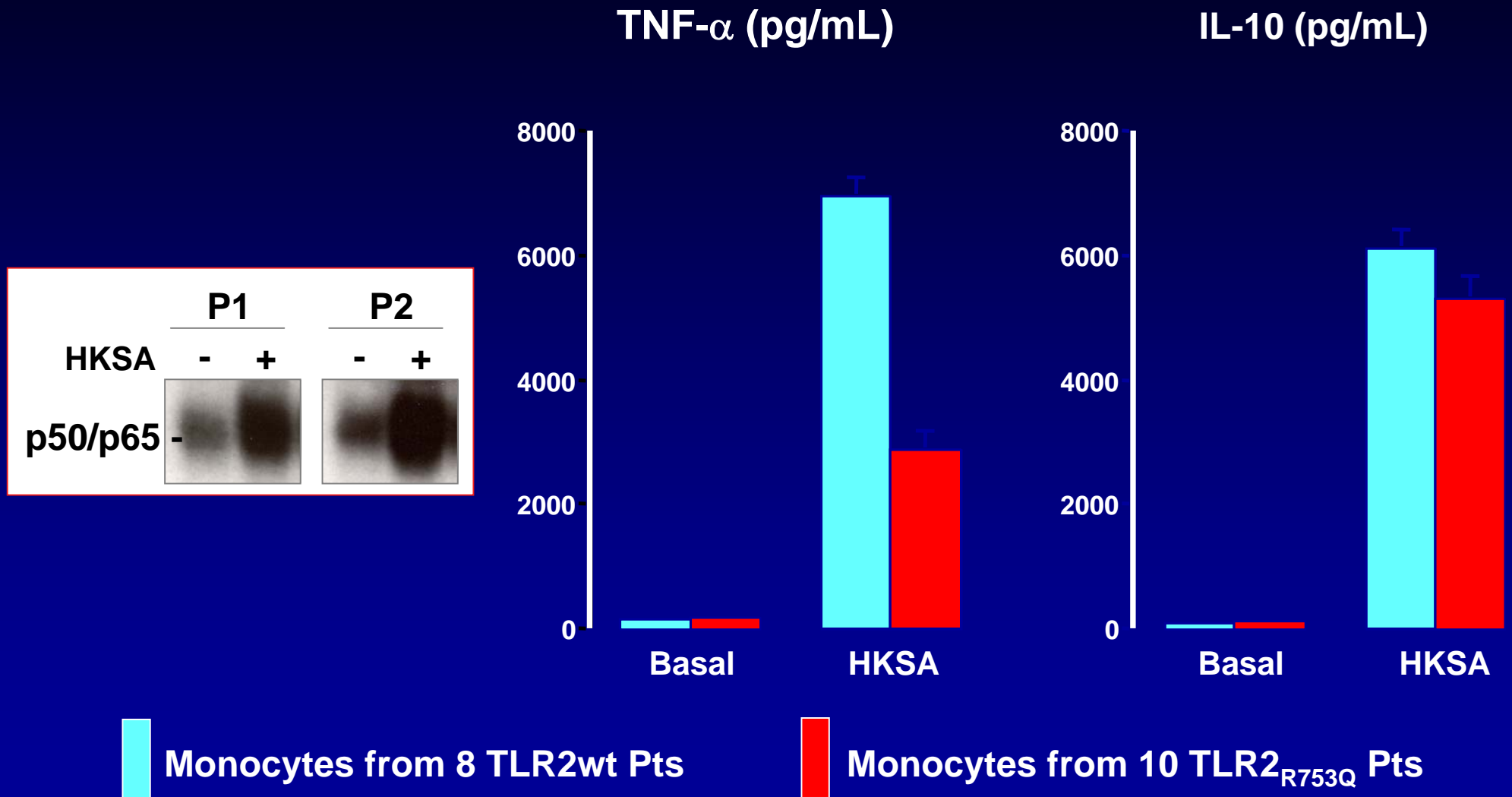
QuickTime™ et un décompresseur  
Cinepak sont requis pour visualiser  
cette image.

*TLR2 stably-transfected 293 cells*  
*GFP-AKT-PH*  
*HKSA*

# TLR2 Polymorphisms in Humans



# TLR2<sub>R753Q</sub> and Cytokine Production



# TLR2<sub>R753Q</sub> in MICU

1103 Caucasian ICU Pts (322 SS) → 28 Pts TLR2<sub>R753Q</sub> (2,5%)

- Age 47,2 ± 18
- SAPS 2 40,5 ± 19
- Survivors 23/28
- Infections 16/28 → 16 Septic Shock (5.1% of SS group)

## • Origin of Infections

- Pneumonia 10
- Meningitis 4
- Septicemia 5
- Others 4

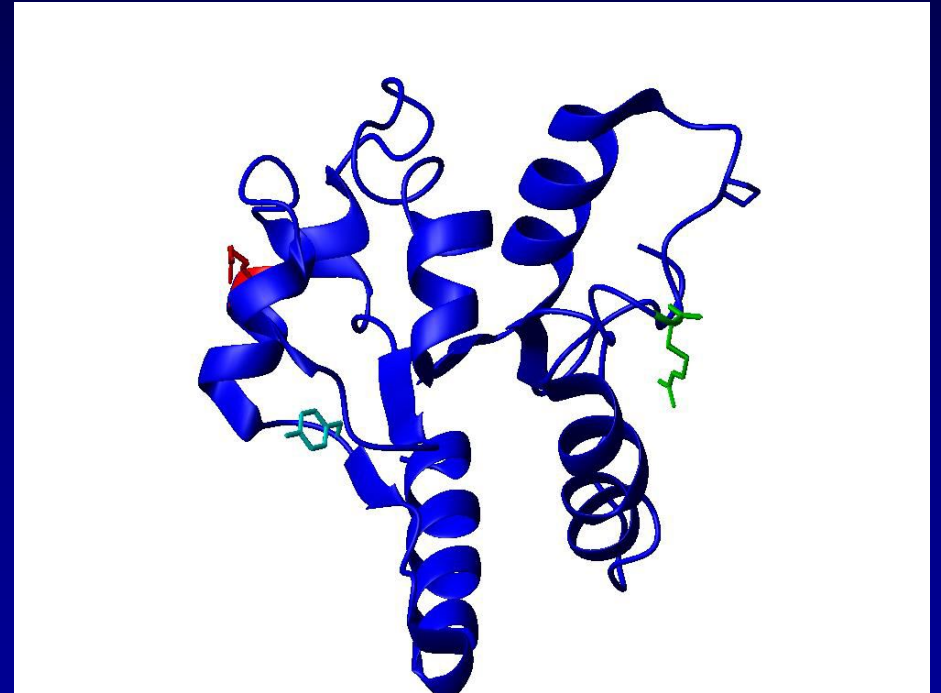
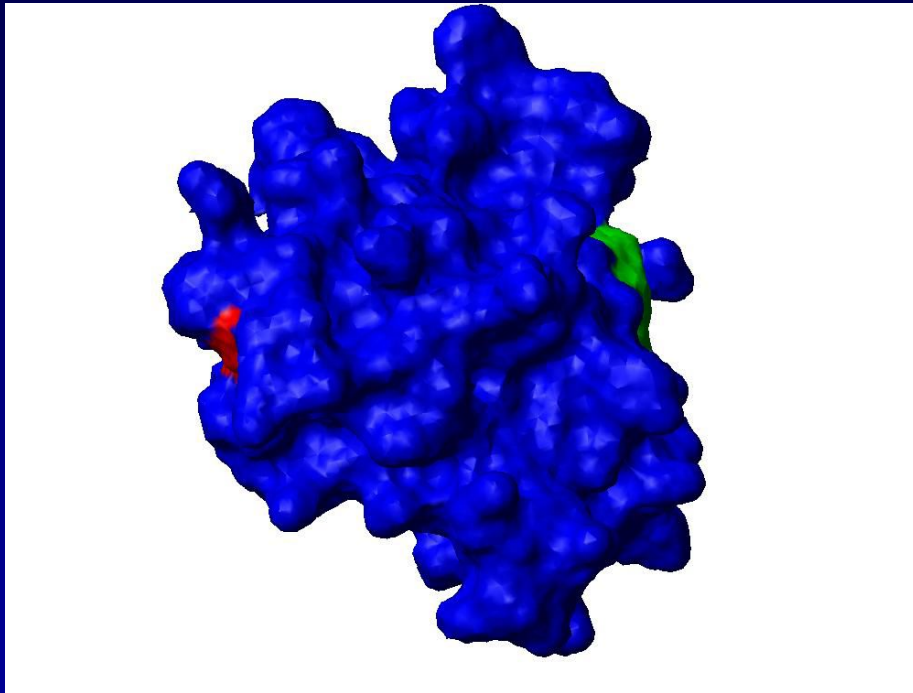
## • Microorganisms

- S. pneumoniae* 8
- S. aureus* 5
- Candida sp.* 2
- Aspergillus sp.* 2
- Others 6

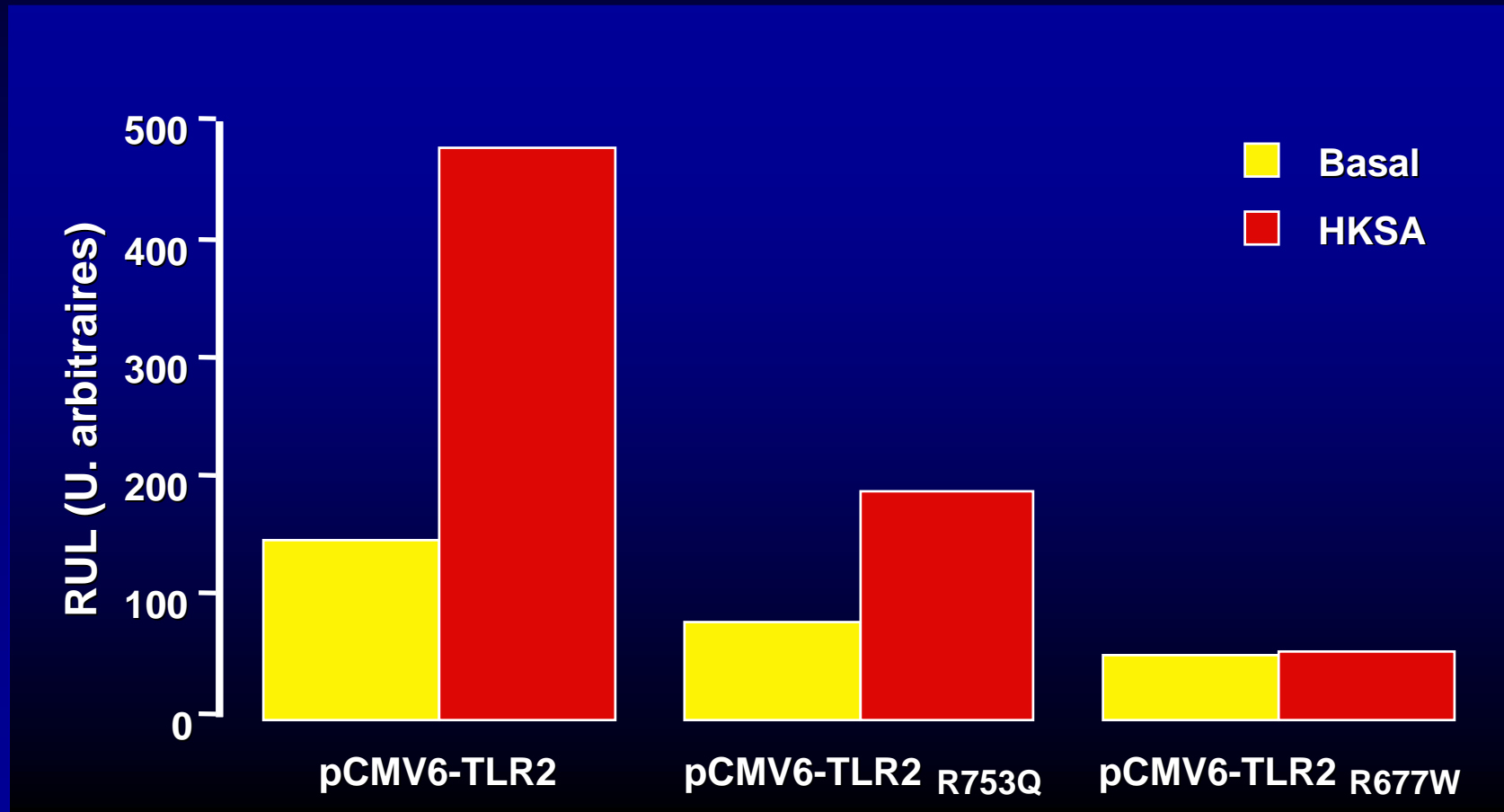
→ 11.1% of Gram positive SS group

# Location of R753 and R677 in the TIR domain

---



# R753Q & R677W inhibit NF-kB activation



# Clinical Importance of TLR2<sub>R677W</sub>

---

## Association of TLR2 R677W with:

- susceptibility to tuberculosis in Tunisian patients

*Ben Ali M; Clin Diagn Lab Immunol. 2004;11:625-6*

- susceptibility to Lepromatous Leprosy

*Kang TJ, FEMS Immunol Med Microbiol. 2001;31:53-8.*

## No association of TLR R677W with:

- chronic mucocutaneous candidiasis

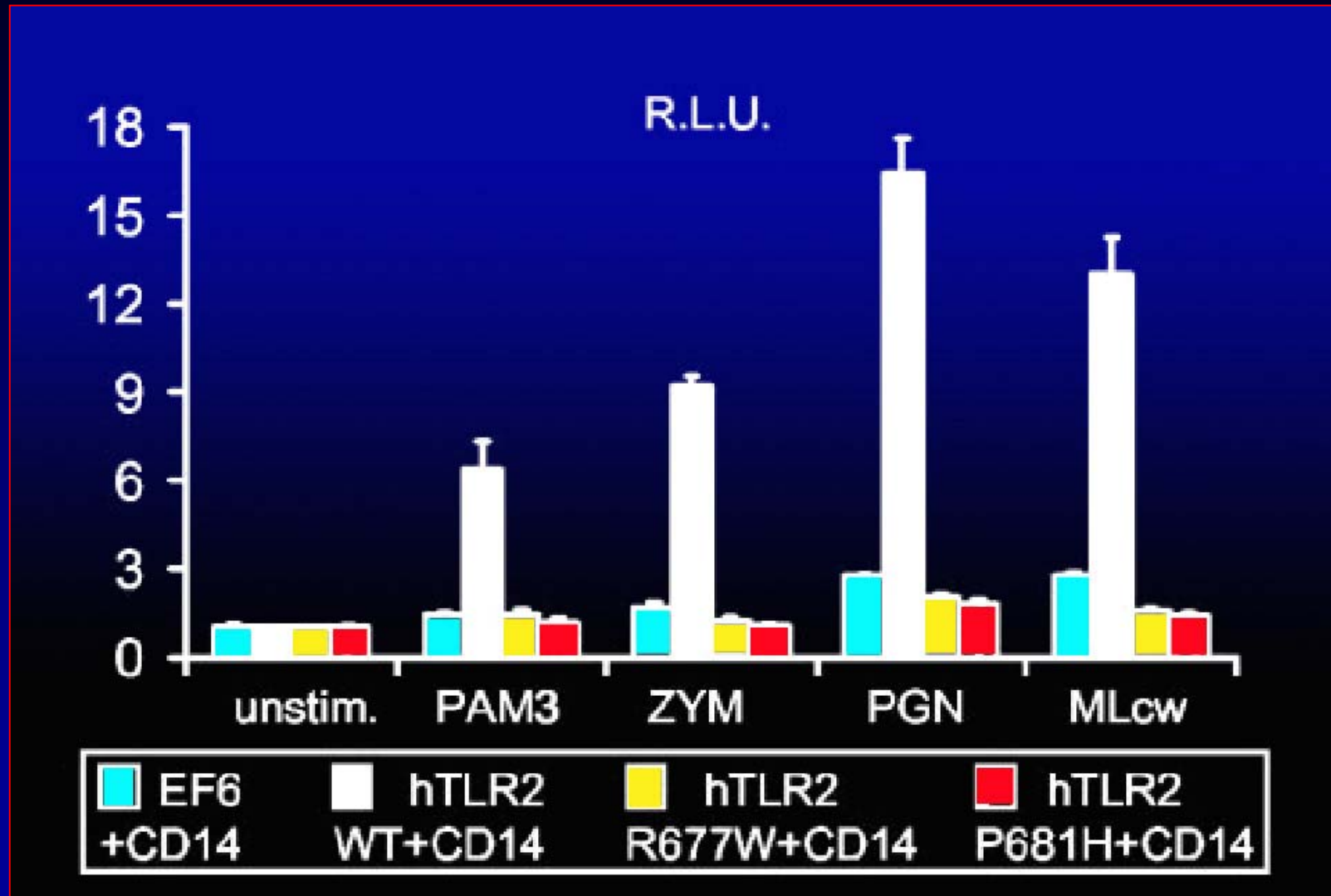
*van der Graaf CA, Neth J med 2003;61:365-9*

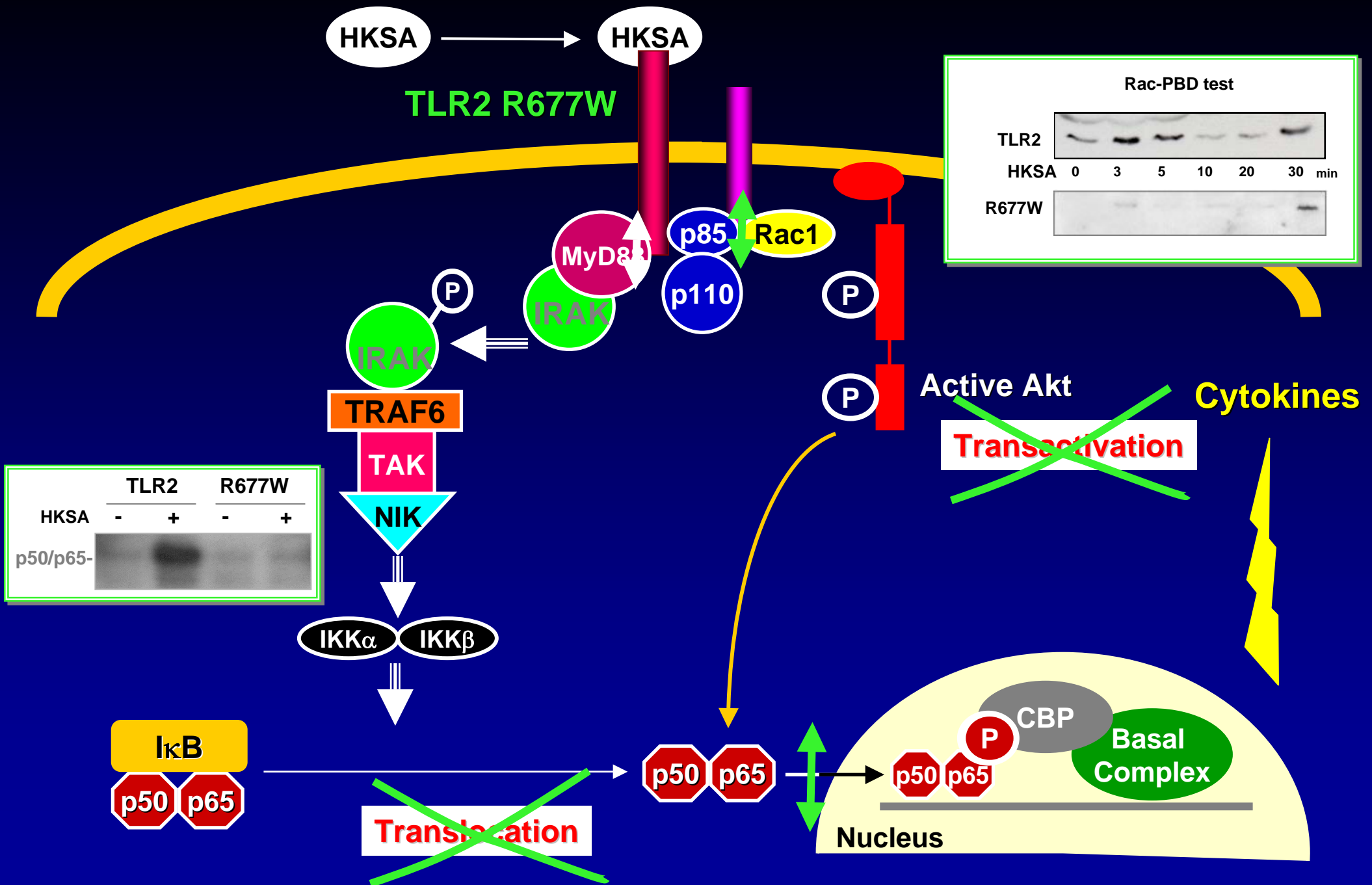
- lymphatic filariasis

*Hise AG, Genes Immun 2003; 4:524-7*

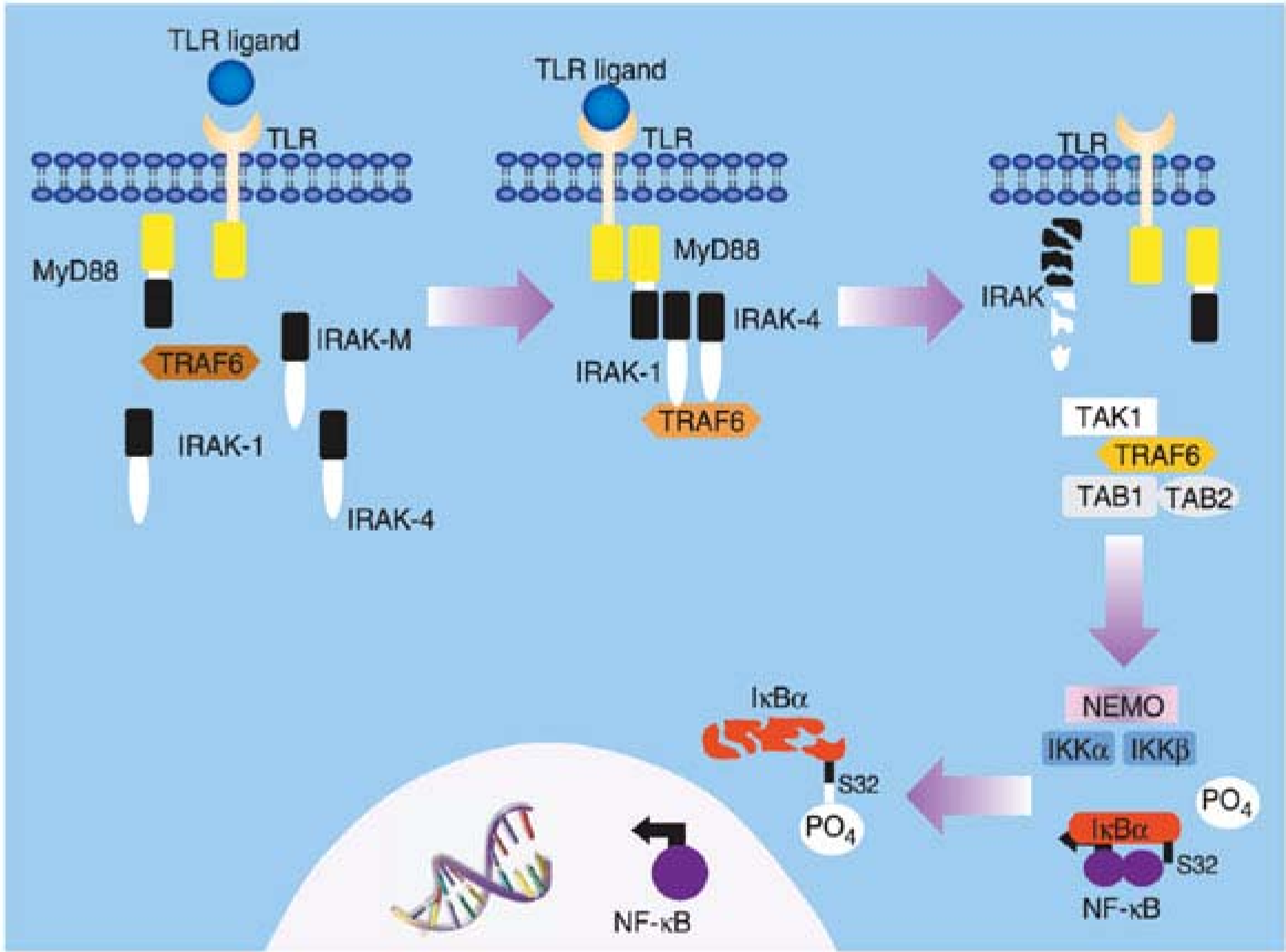


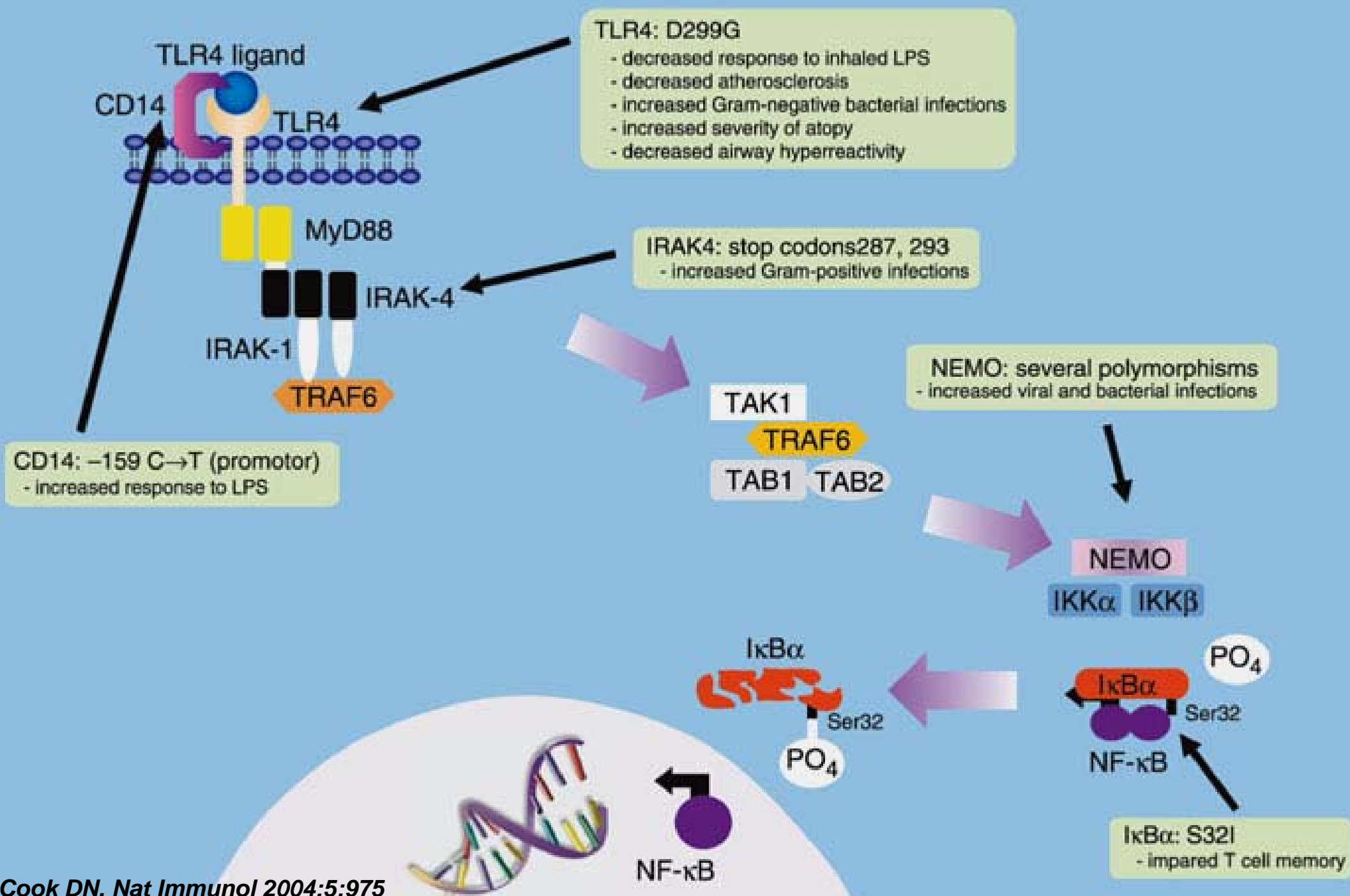
# Polymorphism<sub>R677W</sub> in the *TLR2* gene





# Polymorphisms of TLR Signalling Proteins





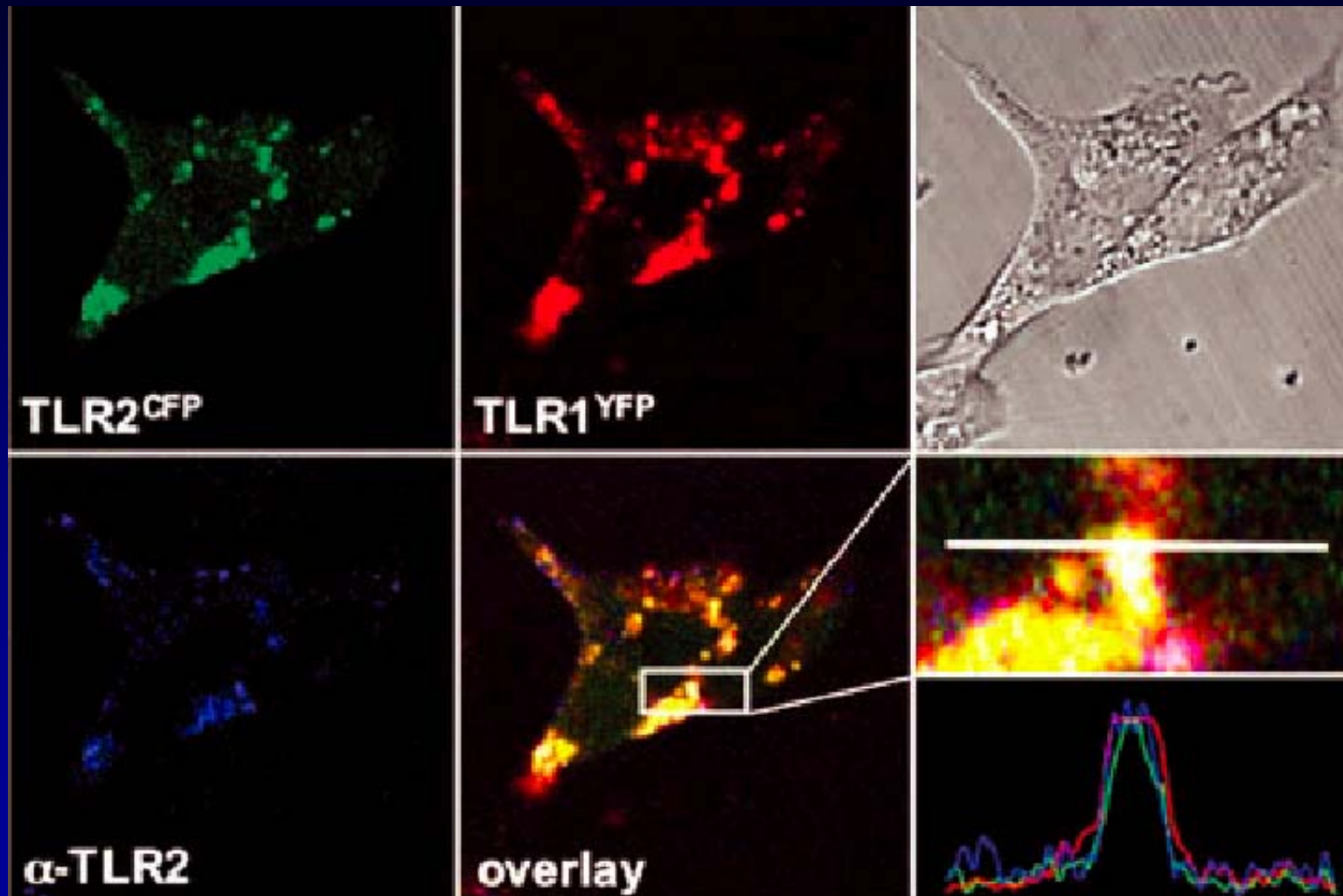
# Conclusions and Perspectives

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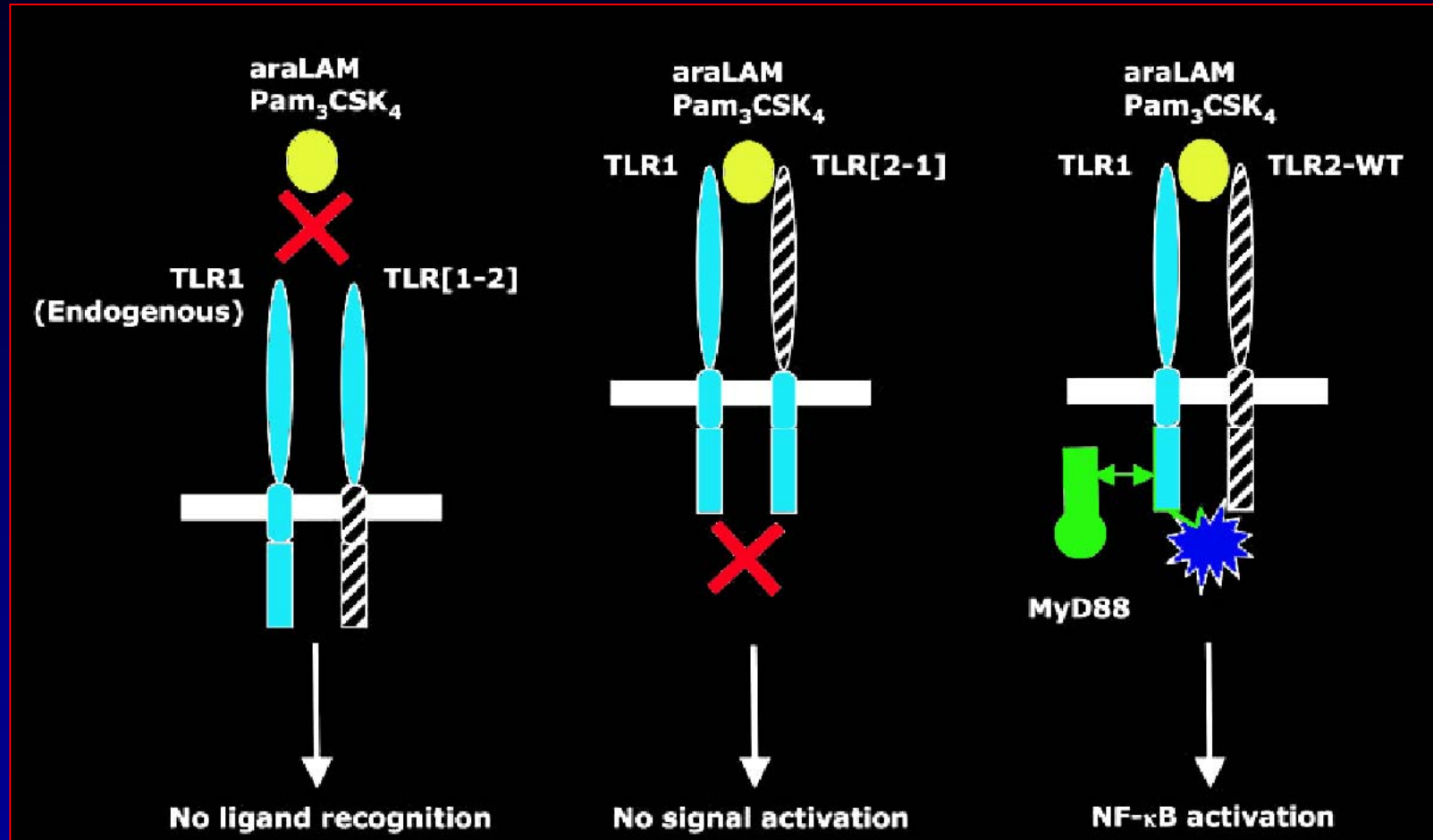
- **TLR functional genomics might explain some inter-individual variabilities of innate immune response**
- **Functional genomics validates signal transduction in Humans**
- **Importance of TLR1 and TLR6 polymorphisms on TLR2 signalling**

# Surface co-localisation of TLR1 and TLR2

## Surface antibody-patching of TLR2



# Complementarities of TLR2 and TLR1 for signalling



TLR1: 77 SNPs

TLR6: 53 SNPs



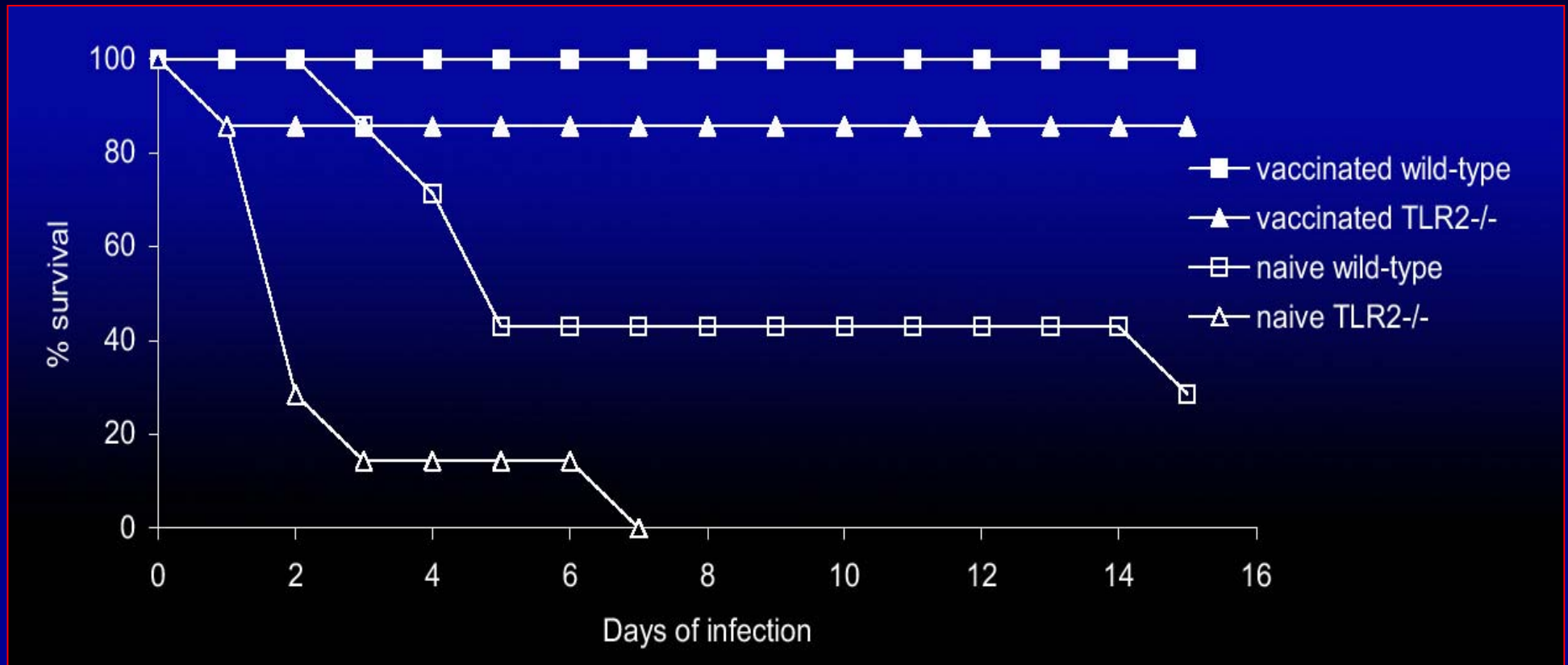
# Conclusions and Perspectives

---

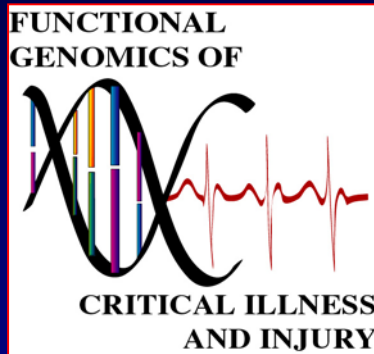
- TLR functional genomics might explain inter-individual variability of innate immune response
- Functional genomics validates signal transduction in Humans
- Importance of TLR1 and TLR6 polymorphisms on TLR2 signalling
- **TLR2 polymorphism detection for severe sepsis prevention**

# Immunization of TLR2-deficient mice

IV infection with  $10^6$  virulent *C. albicans*

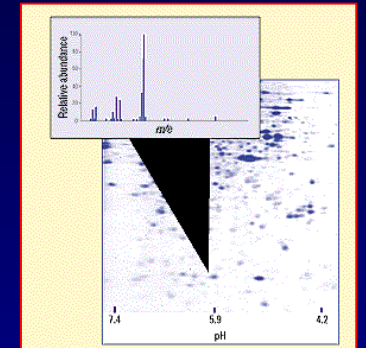


# Clinique



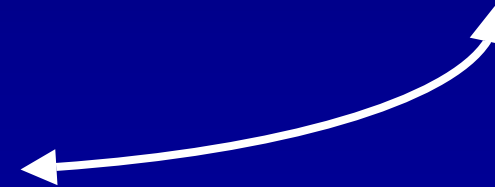
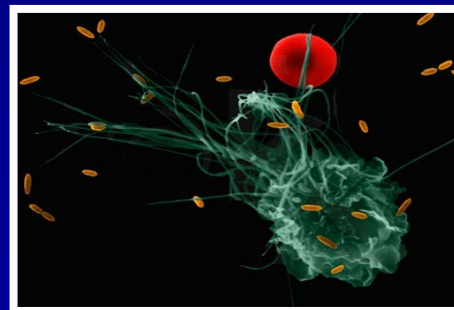
**Diagnostic    Pronostic    Therapeutic**

**MARKERS**



# Génomique

# Proteomic



# Biology

# Acknowledgments

## Cochin Institute - Paris

CHICHE Jean-Daniel

DHAINAUT Jean-François

TEXEREAU Joelle

GRIMALDI David

ROGET Karine

ROUSSEAU Christophe

COMBA Béatrice

CHRABIEH Maya

## Collaborations

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