Genetic Factors Governing Susceptibilities to Severe Infections

GSK-Chair of Infectious Diseases

Pr Jean-Paul MIRA

Sommes-nous tous égaux devant les infections graves?

Université catholique de Louvain
Ecole de médecine
18 Février 2005

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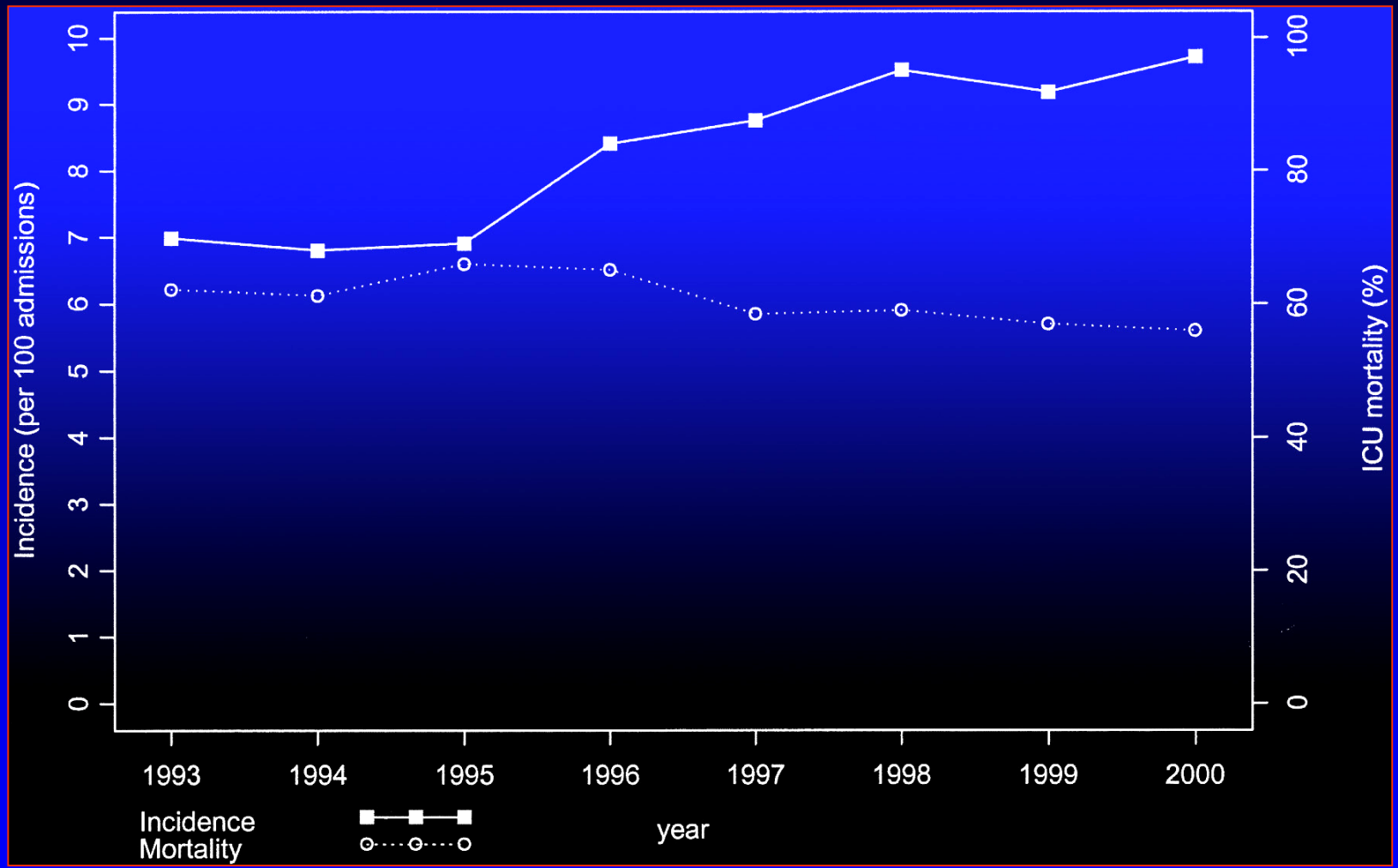
Hôpital Cochin & Institut Cochin, Paris

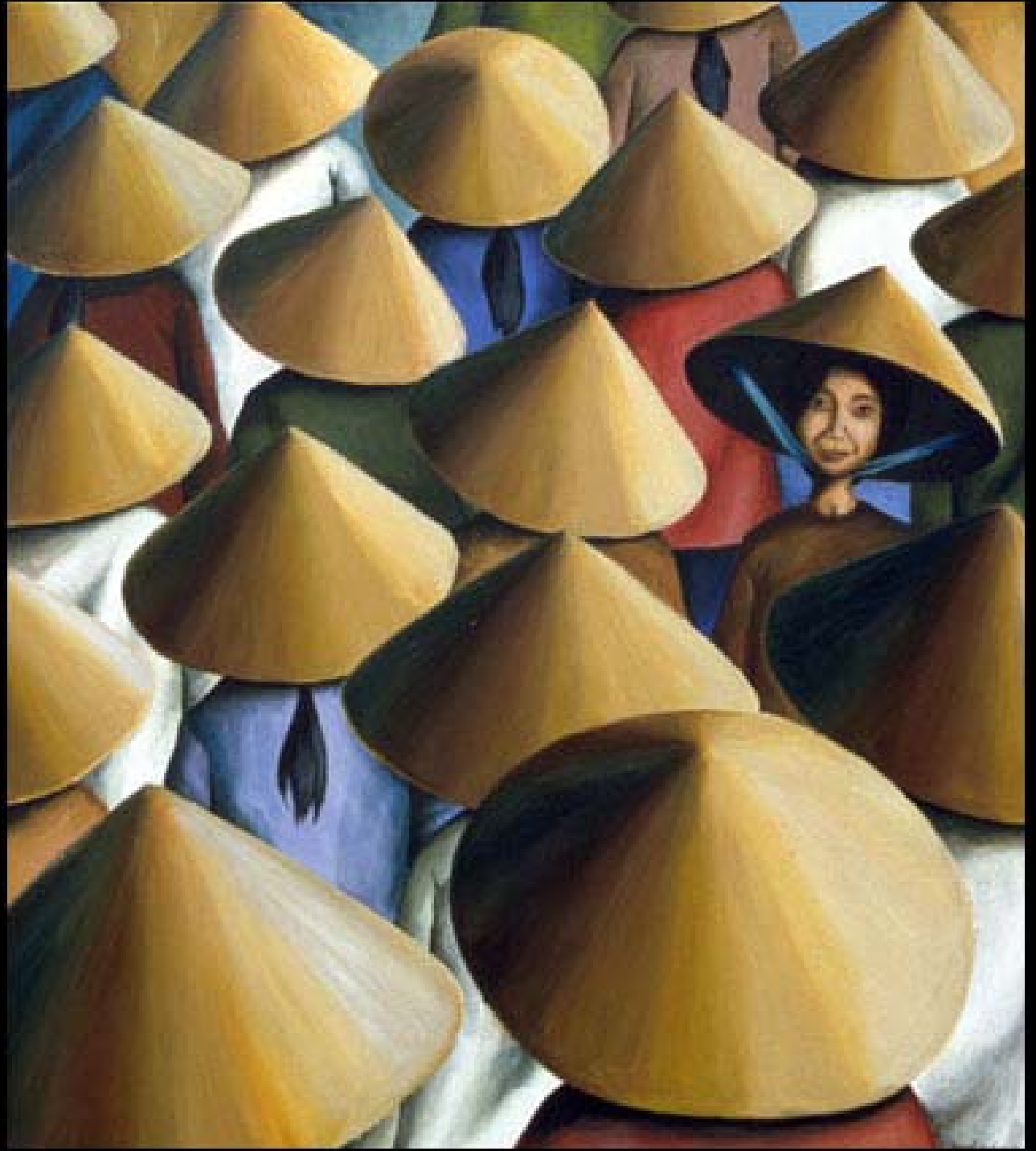
SEPSIS

- Major Cause of Mortality
  - 1st cause of death in ICU
- 1,5 million of severe sepsis/year (Europe)
  - 9% of ICU admissions
  - Estimated cost : 17 milliards $/year (USA)
  - Increased incidence over the years

Septic Shock Epidemiology

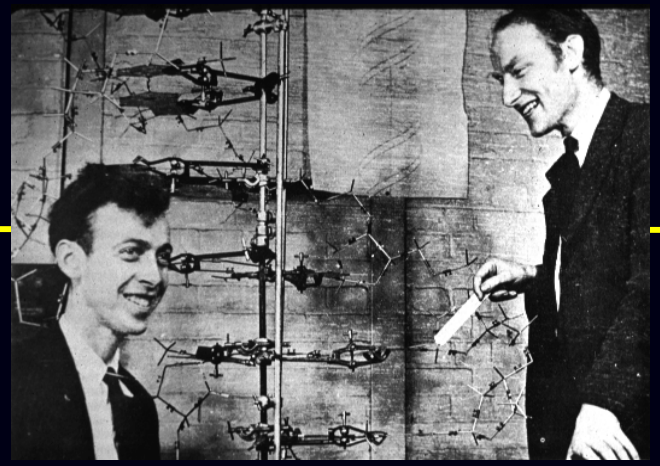


Annane D. Am J Respir Crit Care Med 2003;168:165–172



« 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

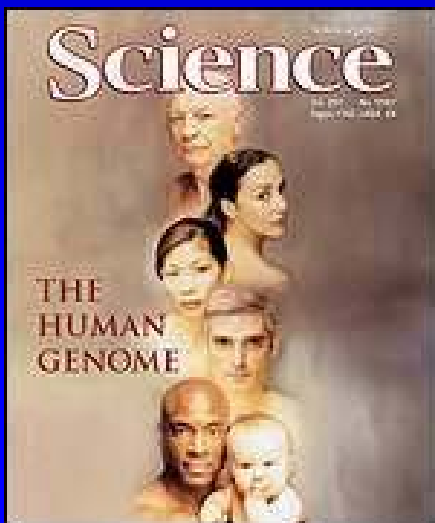
2001-2003

« 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

From Watson and Crick to Human Genome

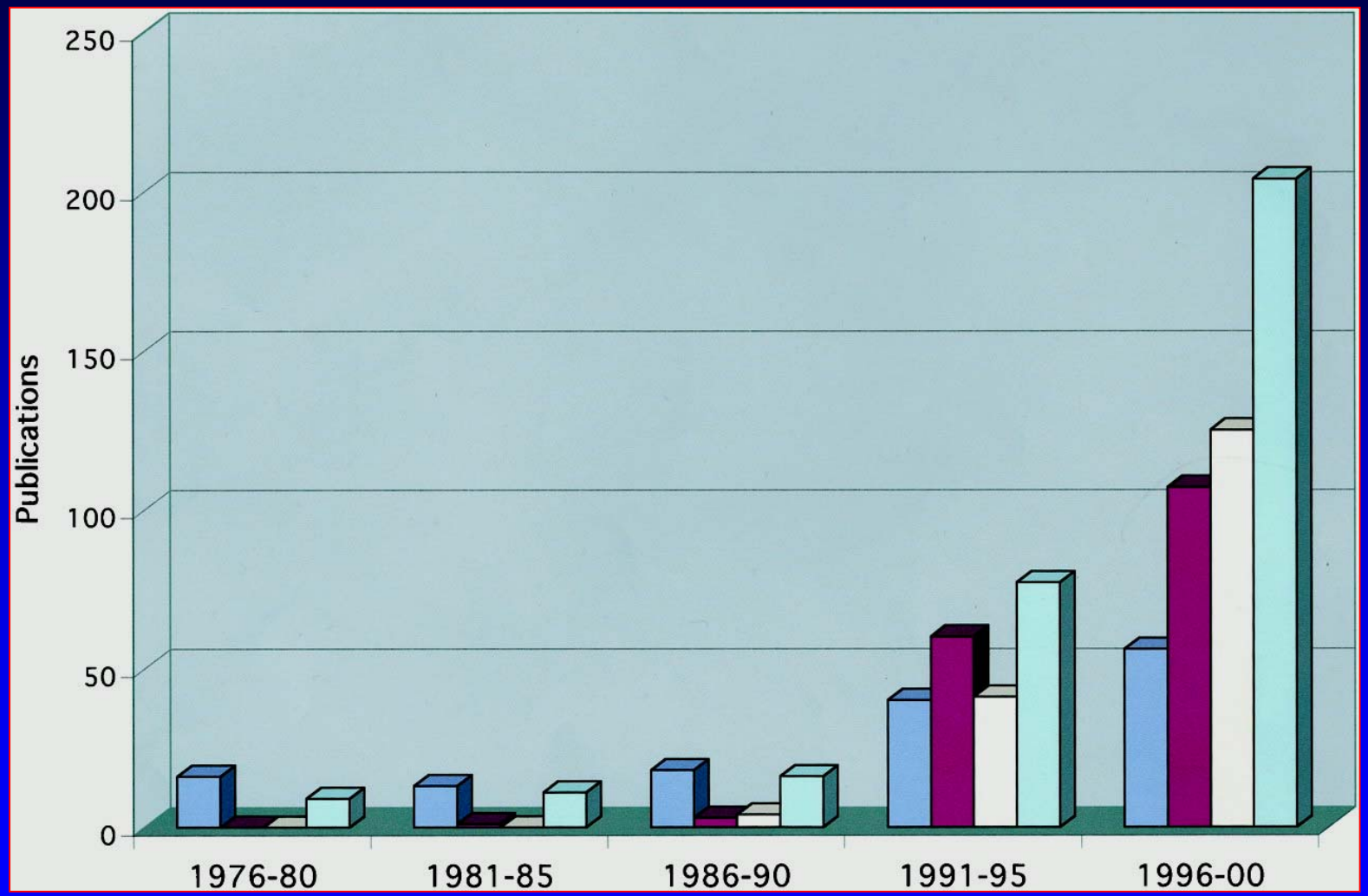
1953	Watson and Crick: double helical structure of DNA
1960s	Role of RNA and Genetic Code
1970s	Recombinant DNA technology
1977	Sanger and Gilbert: DNA sequencing
1983	Mapping of disorders by linkage (Huntington disease)
1986	Polymerase Chain Reaction
1990	Human Genome Project
1995	*Haemophilus influenzae* genome
2003	Mice and Human genome sequence
	Human SNP Map




La Recherche
Génome humain
Les vrais enjeux d'un grand programme


TIME
LOTTE'S IMPEACHMENT PLAN
SPECIAL ISSUE
THE FUTURE OF MEDICINE
How genetic engineering will change us in the next century

Molecular Genetic Research



Milewitcz DM. Circulation 2000; 102: IV-103


TOUS LES ÊTRES HUMAINS
PARTAGENT LES MÊMES GÈNES
MAIS...

Small differences in genotype make big differences to phenotype


DNA
EVIDENCE

Evidences for a genetic component to sepsis

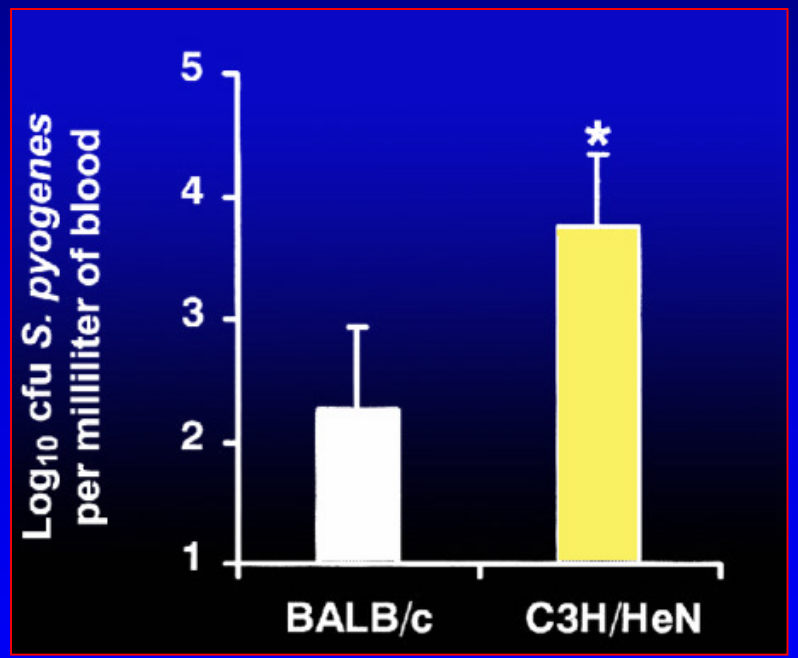
Animal Studies

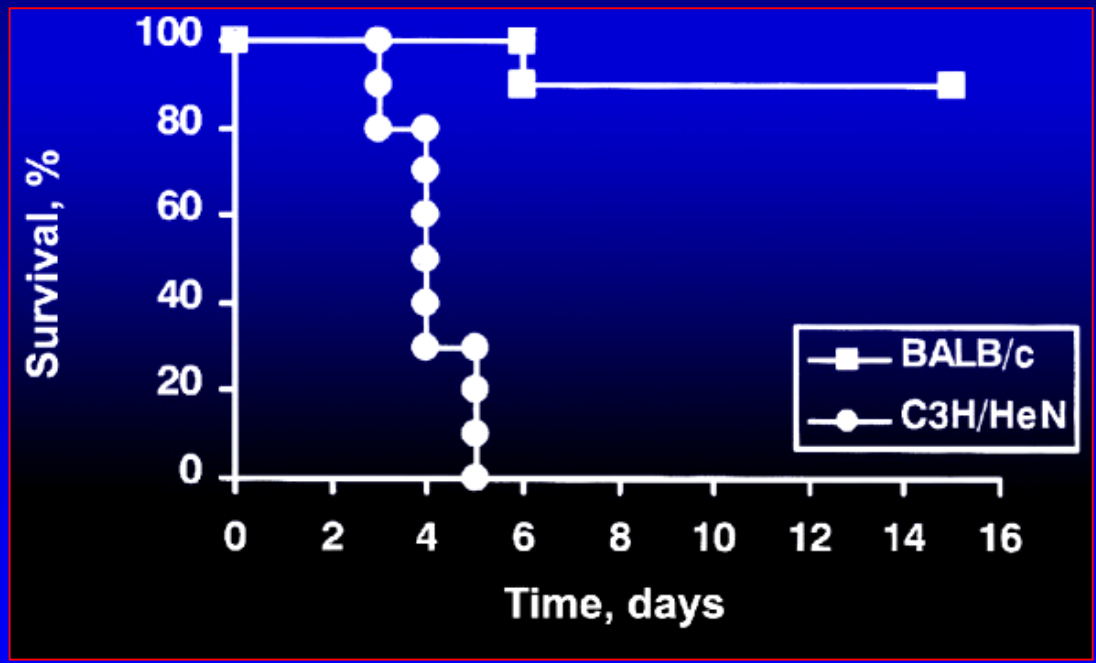
- Susceptibility/resistance to certain infection in mice

- Susceptibility/resistance phenotypes of knock-out mice

Mice Susceptibility to Infection with Group A Streptococci

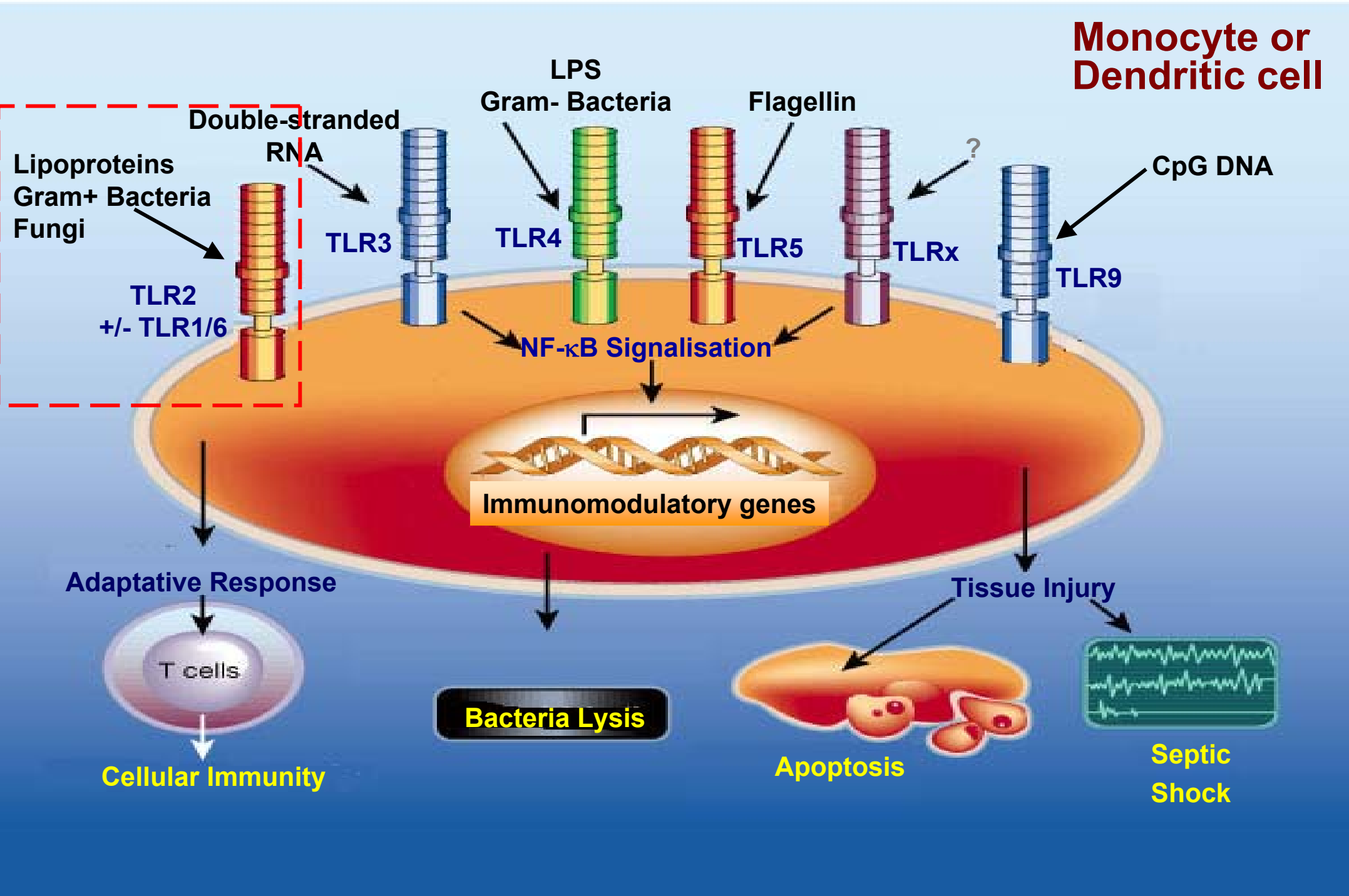
10^3 cfu *Strepto* Subcutaneous





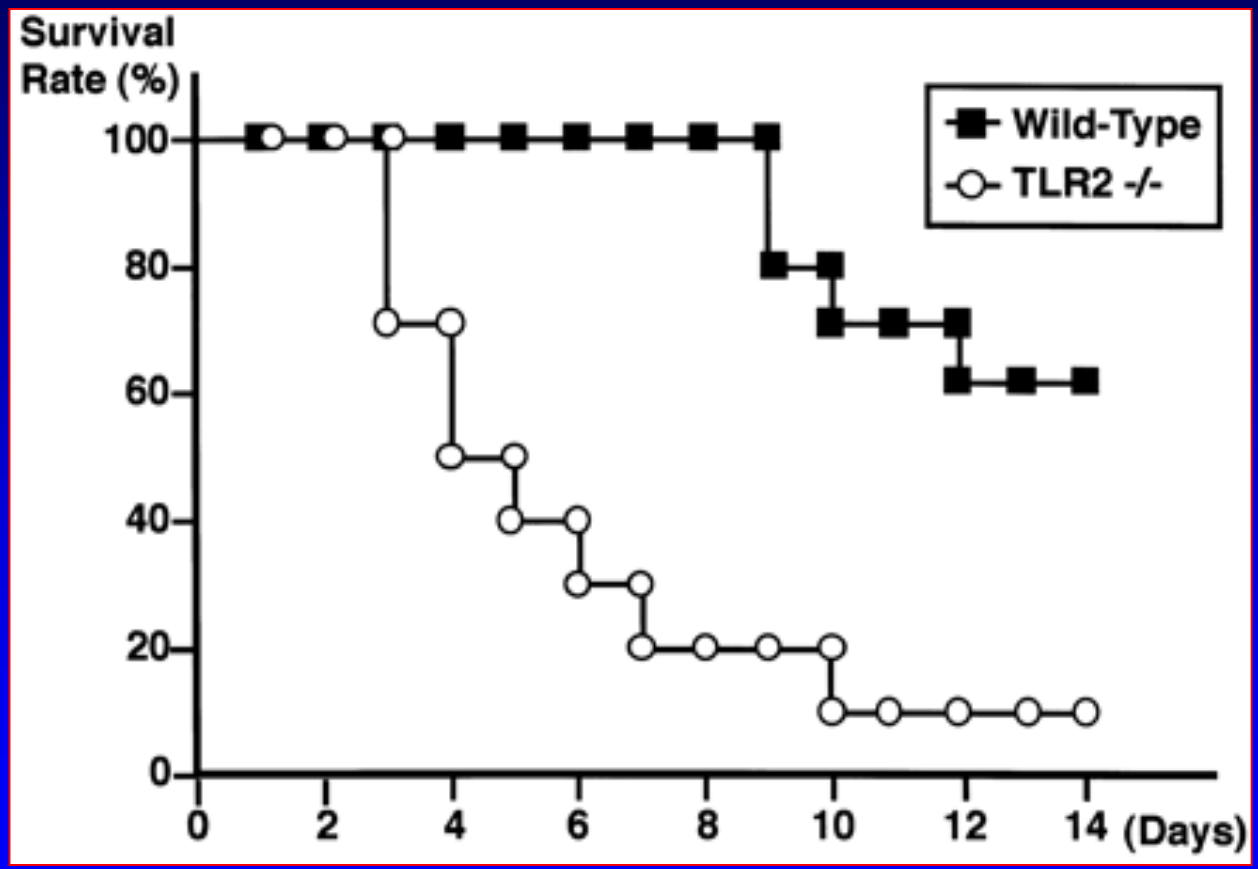
d2

Goldman O. J Infect Dis 2003;187:854-61.


Monocyte or
Dendritic cell
LPS
Gram- Bacteria
Flagellin
Double-stranded
RNA
Lipoproteins
Gram+ Bacteria
Fungi
?
CpG DNA
TLR3
TLR4
TLR5
TLRx
TLR9
TLR2
+/- TLR1/6
NF-κB Signalisation
Immunomodulatory genes
Adaptative Response
T cells
Cellular Immunity
Bacteria Lysis
Tissue Injury
Apoptosis
Septic
Shock

TLR2-KO Mice and Response to Gram Positive Bacteria

Intraveneous infusion of Staphylococcus aureus



Takeuchi et al., J. Immunol. 1999; 165:5392

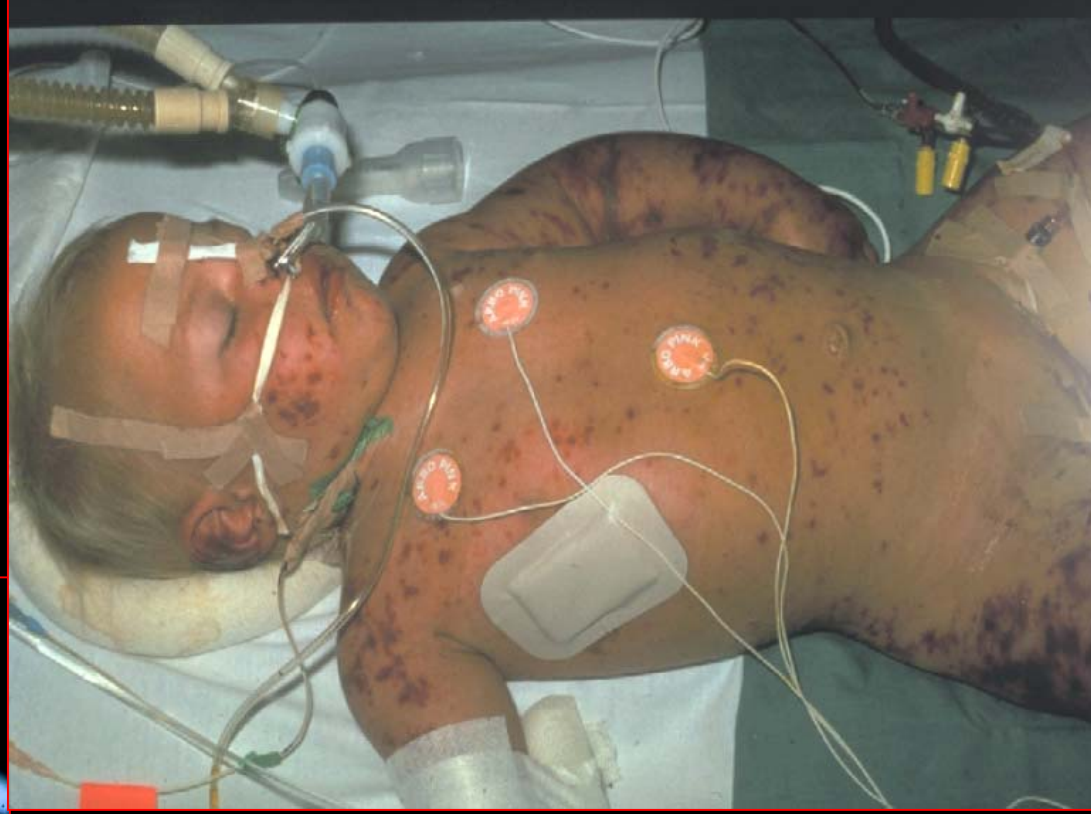
Evidences for a genetic component to sepsis

Animal Studies

- Susceptibility/resistance to certain infection in mice
- Susceptibility/resistance phenotypes of knockout mice

Human Studies

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



Twin Studies

- **Tuberculosis**

Kallmann FJ, Am rev Tuber 1943.
Comstock GW, Am Rev Respir Dis 1978.

- **Leprosis**

Fine PE, Int J leprosy 1981

- **Helicobacter pylori**

Malaty HM, Ann Intern Med 1994.

- **Malaria**

Jepson AP, J Infect Dis 1995.

- **AIDS**

Chang J, J Virol 1996.

Genetic and environmental influences on premature death in adult adoptees

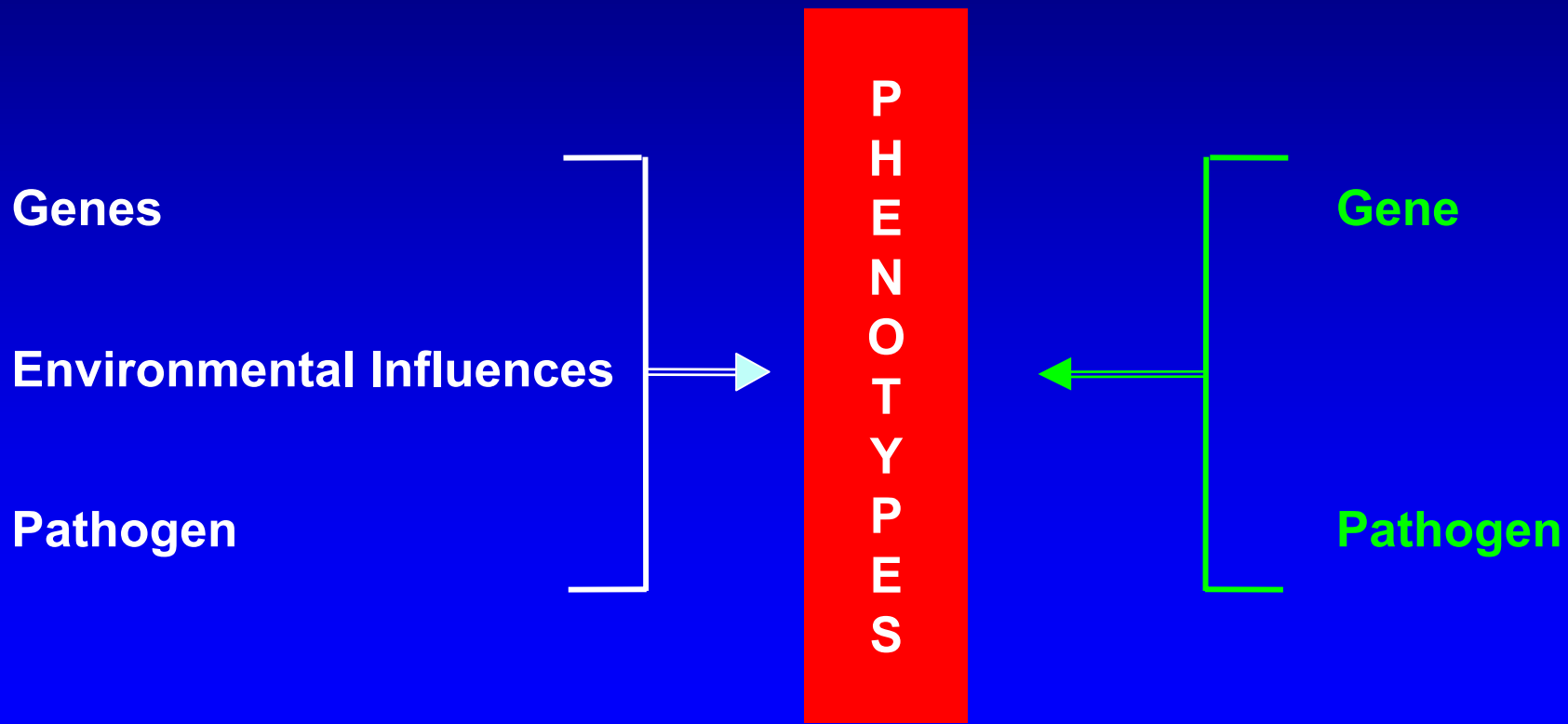
Cause of Death (Parent Dead before the age of 50)	Relative risk for the adoptee to die from the same cause
All causes	
Biologic	**1.71**
Adoptive	**0.71**
Infection	
Biologic	**5.8**
Adoptive	**0.73**
Vascular	
Biologic	**4.5**
Adoptive	**3.1**

Sørensen TI, et al. NEJM 1988; 318:727-32.

Host Genetics of Infectious Diseases

Genetics of Complex Diseases

Mendelian Genetics



Genetics of Phagocyte Immune Defects

Rolling Deficiciency

Type II: Sialyl Lewis^X (AR)

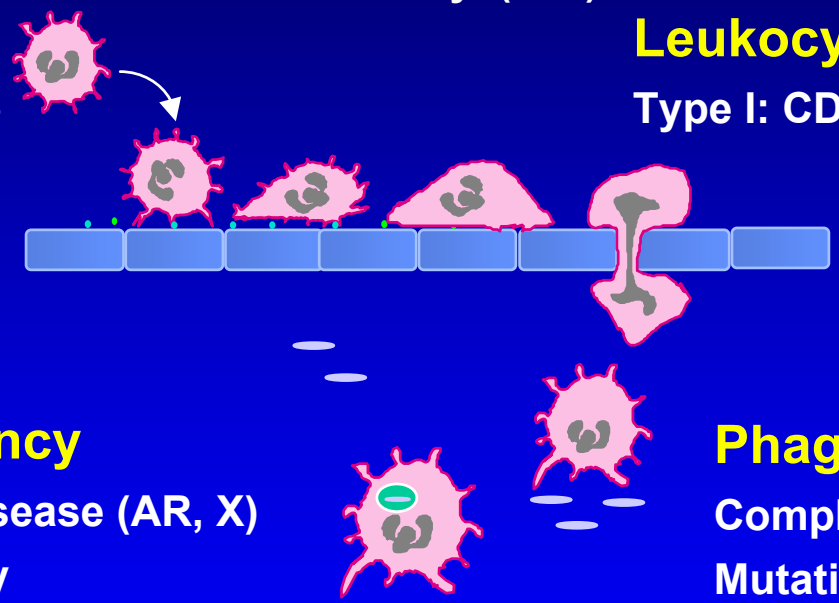
Selectin Deficiency (AR)

Number

Cyclic neutropenia (AD)

Congenital agranulocytosis

(Kostmann syndrome)



Leukocyte Adhesion Deficiency

Type I: CD18 (AR);

Bacterial lysis Deficiency

Chronic Granulomatous Disease (AR, X)

Myeloperoxidase deficiency

Neutrophil granule defects

Phagocytosis Deficiency

Complement deficiencies

Mutations of MBL

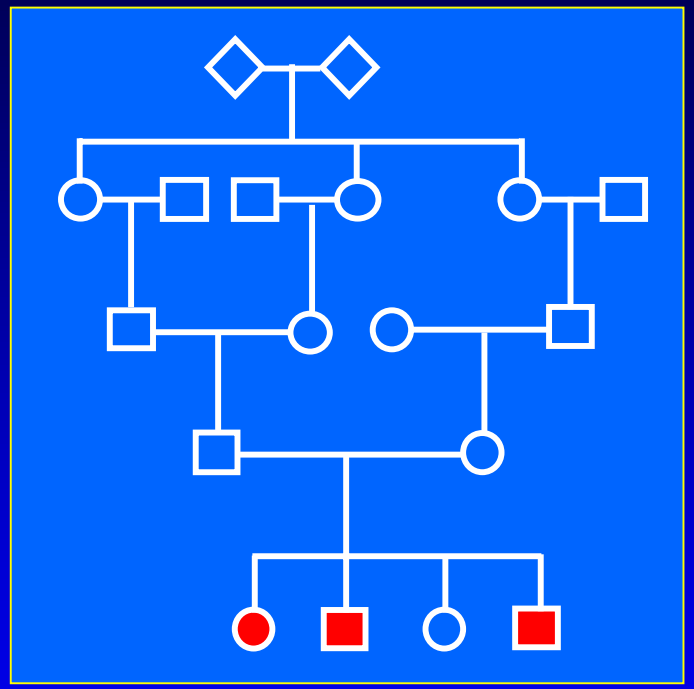
Recurrent Bacterial Infections

Holland SM and Gallin JI, Annu Rev Med 1998

Single Gene Defects and Severe Immunodeficiency Disorders

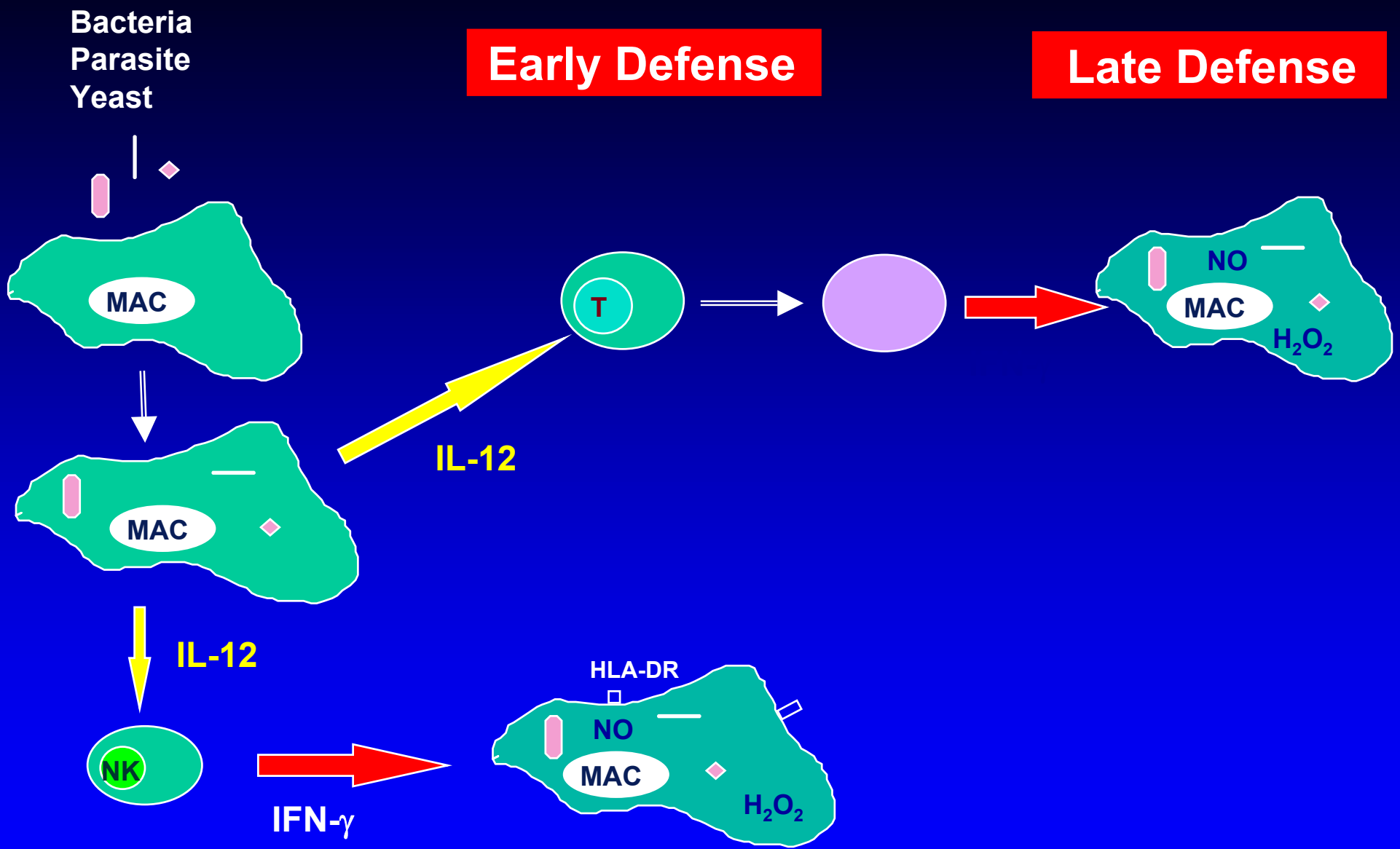
System involved	Typical clinical syndrome	Genetic Defect
B lymphocyte	Defective antibody production	B cell tyrosine kinase CD40 ligand
T lymphocyte	Defective humoral and cellular immunity	IL-2 receptor
Neutrophil	Defective phagocytosis	Cytochrome b β 2 integrin
Macrophage	Susceptibility to mycobacterial infection	Interferon γ receptor
Complement	Recurrent *Neisseria* infections	Terminal complement components

Single Gene Defects and Severe Tuberculosis



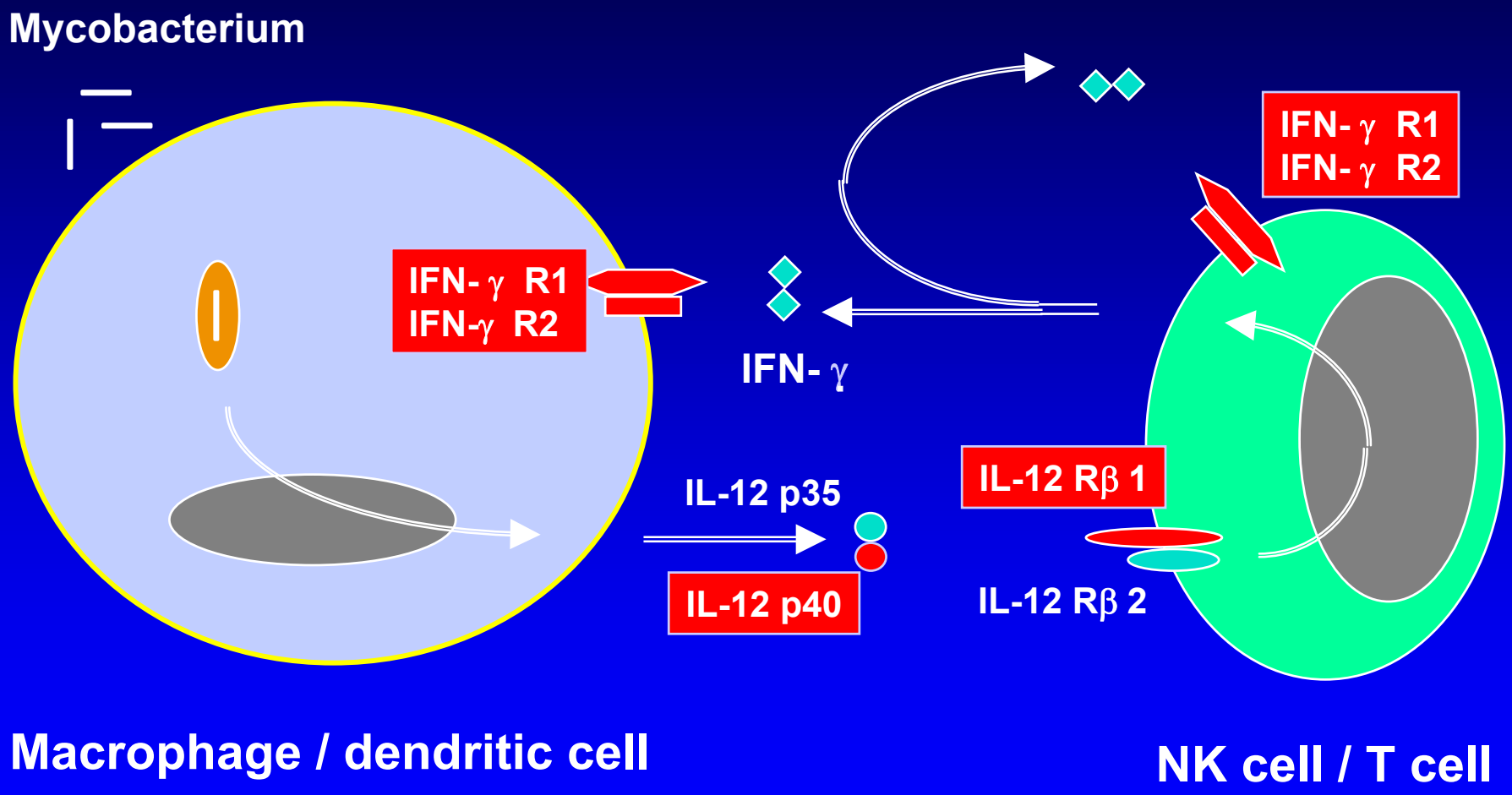
- Parental consanguinity
- Affected siblings
- Familial forms of disseminated infections with weakly pathogenic mycobacteria

Search for recessive genetic disorders

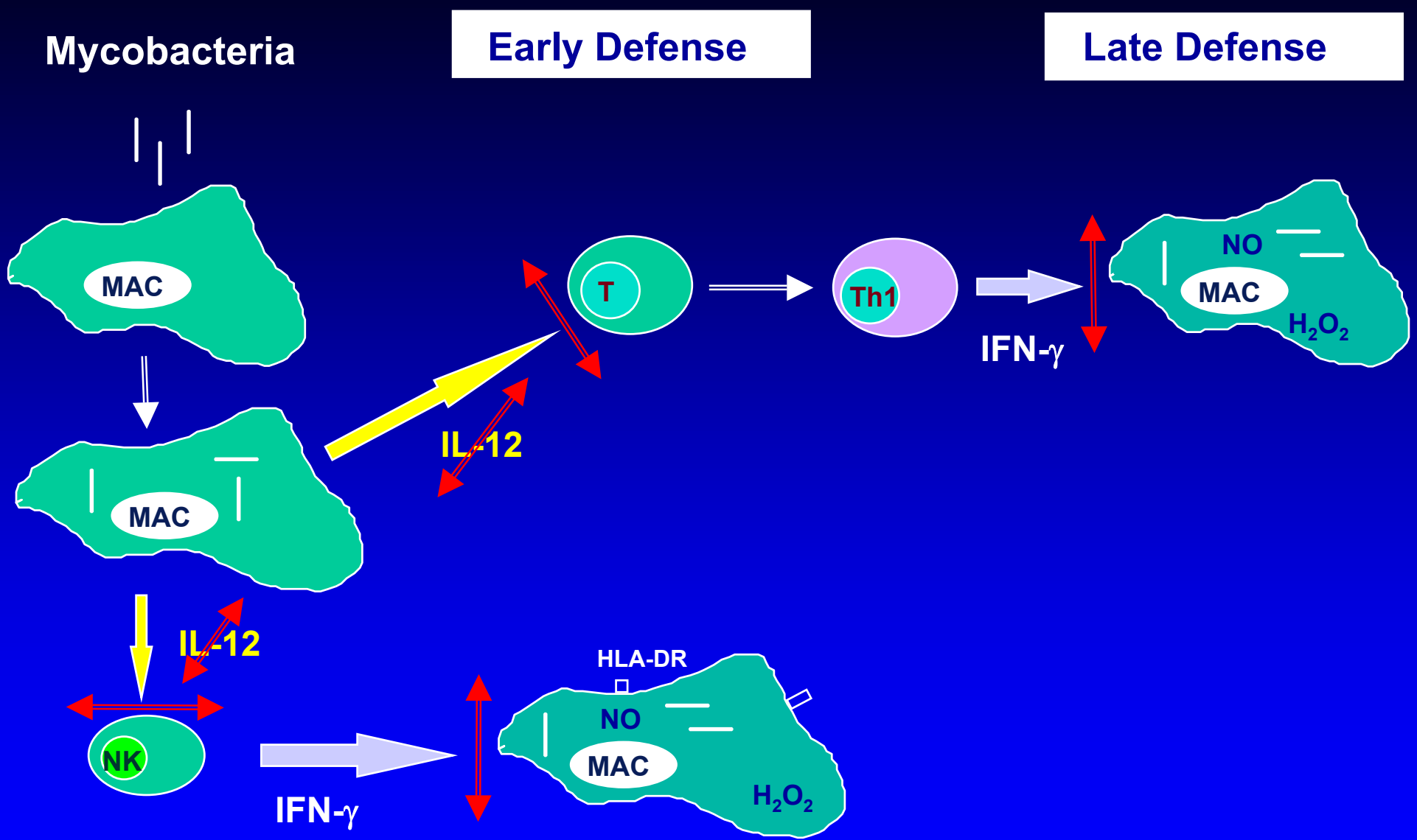


(Biron et Gazzinelli. Curr. Op. Immuno. 1995;7:485-496)

Mendelian susceptibility to mycobacterial infection in man

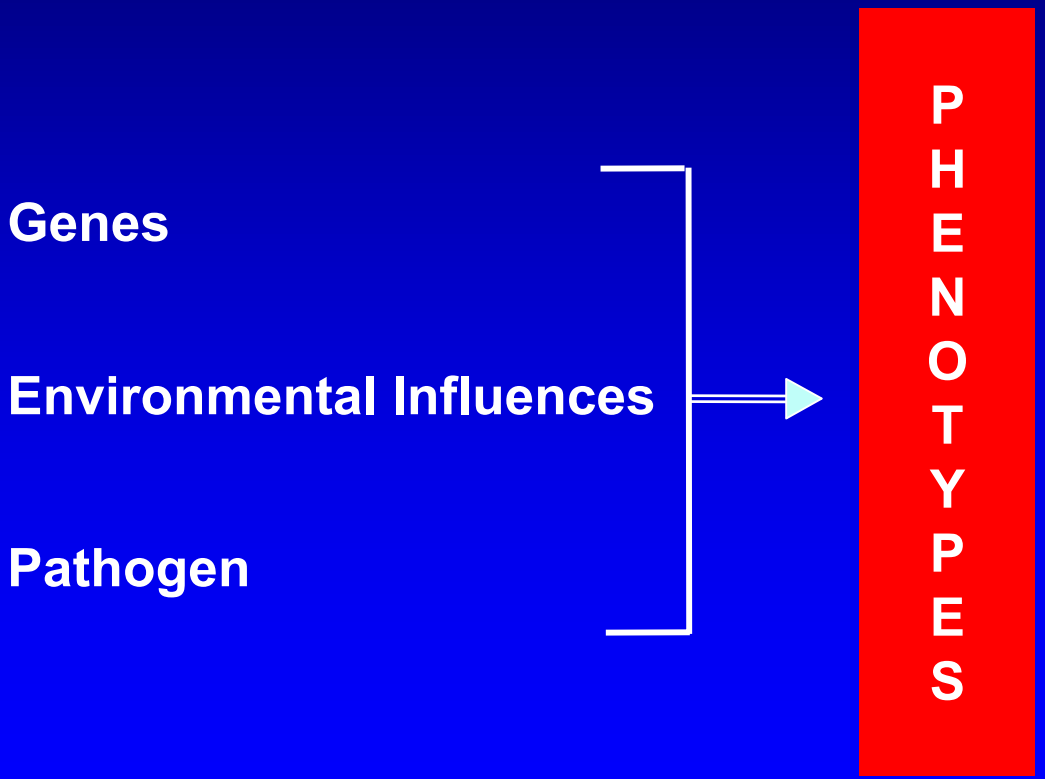


Altare F et al. Curr Op Immunol 1998

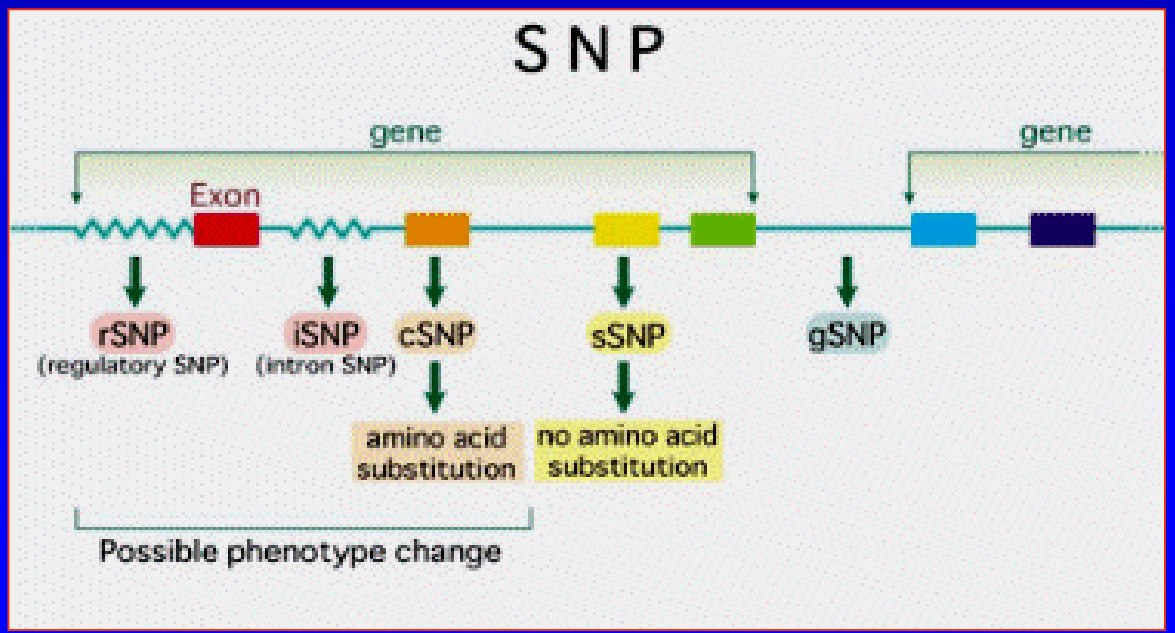

Mycobacteria
Early Defense
Late Defense
MAC
MAC
T
Th1
IL-12
IFN-γ
NO
MAC
H_2O_2
IL-12
NK
IFN-γ
HLA-DR
NO
MAC
H_2O_2

Host Genetics of Infectious Diseases

Genetics of Complex Diseases



Polymorphismes Génétiques



Polymorphismes génétiques fonctionnels

Changement Quantitatif

Détection des pathogènes (CD14; MBL)

Inflammation (TNF; IL-1;…)

Coagulation (PAI-1; VII;…)

Thérapeutique (CYP)

…

Changement Qualitatif

Détection des pathogènes(Toll; FcR;…)

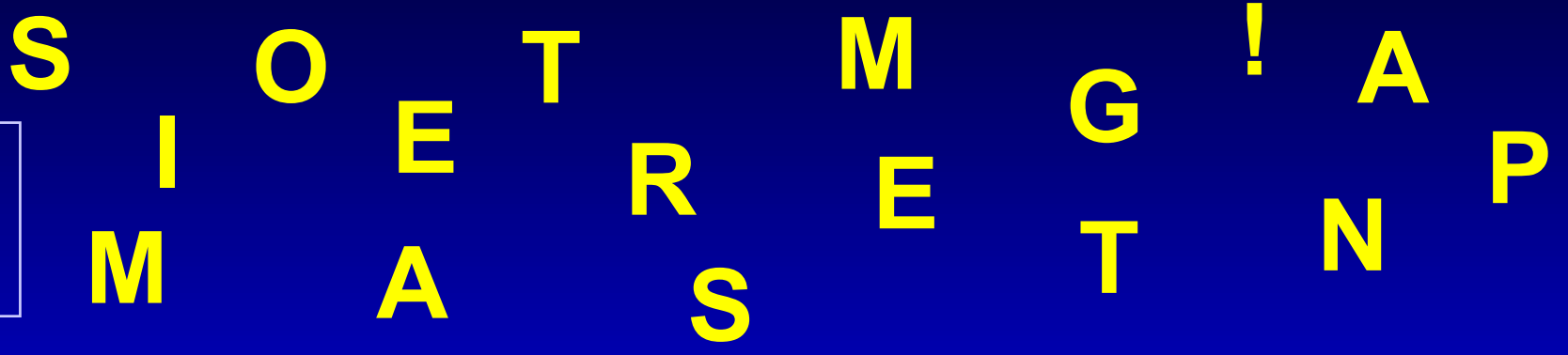
Inflammation (TNFR;iNOS;…)

Coagulation (Leyden;…)

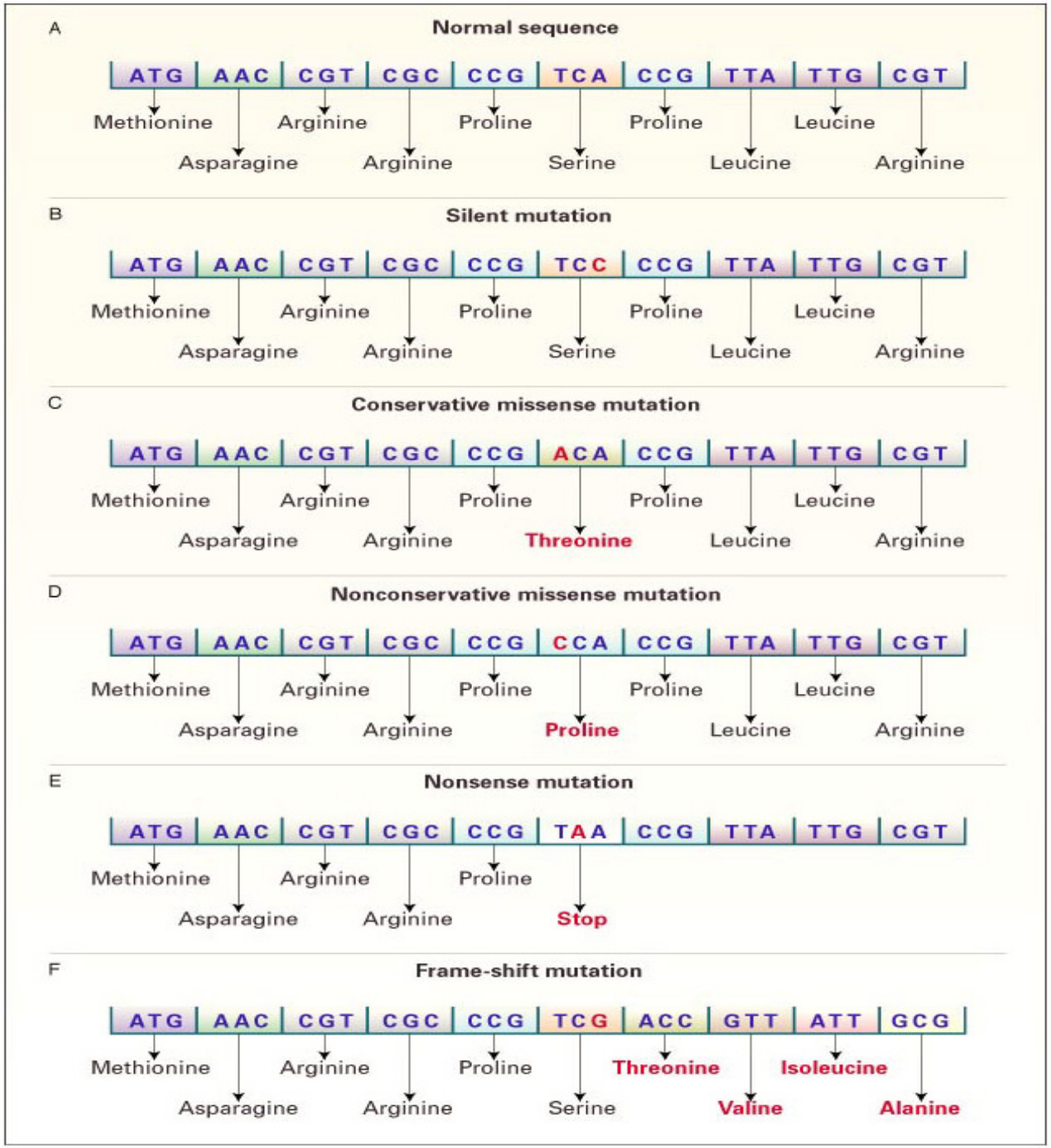
Thérapeutique

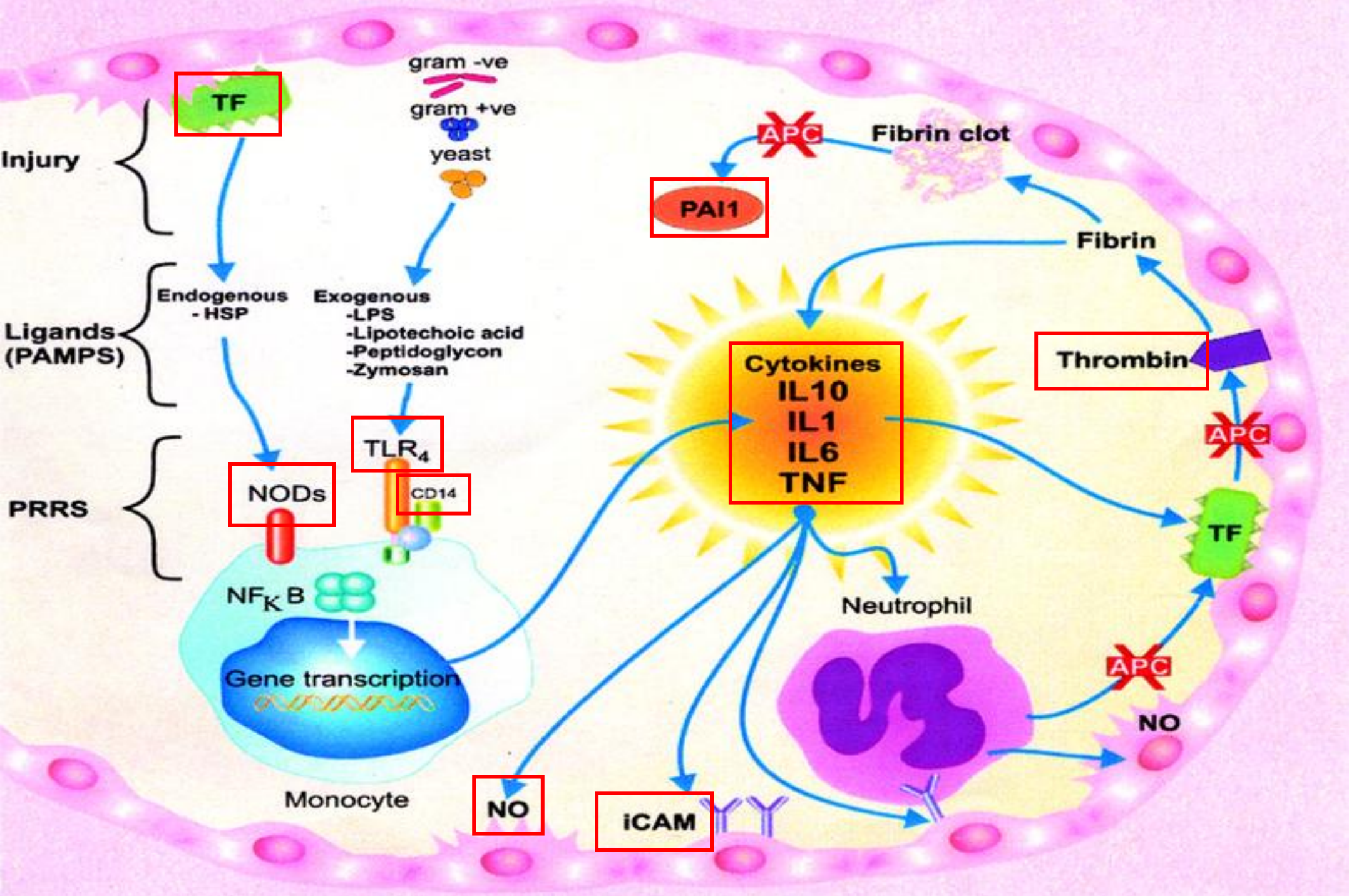
…

Background vs. Functional SNPs



Before DNA Sequencing	
DNA Sequence	IMPORTANT MESSAGE !
Variation 1	IMPERTANT MESSAGE !
Variation 2	*IMPORTANT MASSAGE !*

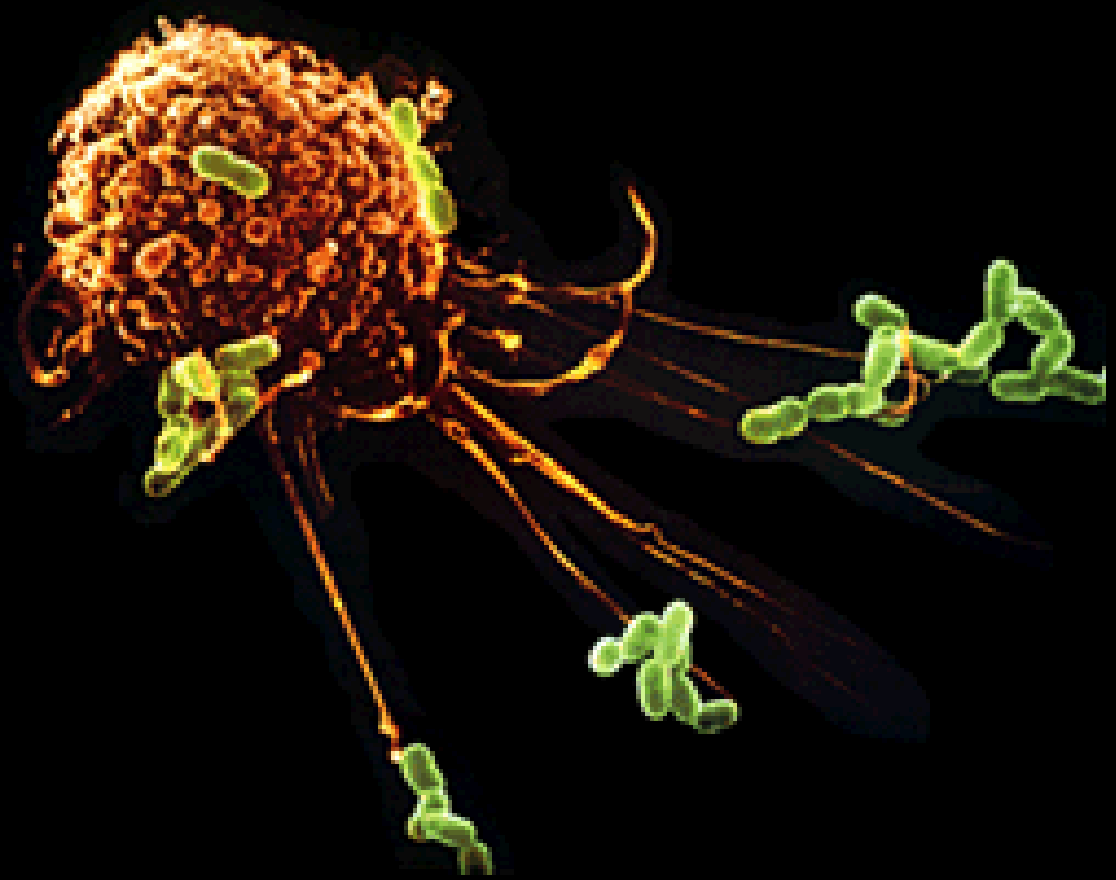

A
Normal sequence
ATG AAC CGT CGC CCG TCA CCG TTA TTG CGT
Methionine Arginine Proline Proline Leucine
Asparagine Arginine Serine Leucine Arginine
B
Silent mutation
ATG AAC CGT CGC CCG TCC CCG TTA TTG CGT
Methionine Arginine Proline Proline Leucine
Asparagine Arginine Serine Leucine Arginine
C
Conservative missense mutation
ATG AAC CGT CGC CCG ACA CCG TTA TTG CGT
Methionine Arginine Proline Proline Leucine
Asparagine Arginine Threonine Leucine Arginine
D
Nonconservative missense mutation
ATG AAC CGT CGC CCG CCA CCG TTA TTG CGT
Methionine Arginine Proline Proline Leucine
Asparagine Arginine Proline Leucine Arginine
E
Nonsense mutation
ATG AAC CGT CGC CCG TAA CCG TTA TTG CGT
Methionine Arginine Proline
Asparagine Arginine Stop
F
Frame-shift mutation
ATG AAC CGT CGC CCG TCG ACC GTT ATT GCG
Methionine Arginine Proline Threonine Isoleucine
Asparagine Arginine Serine Valine Alanine

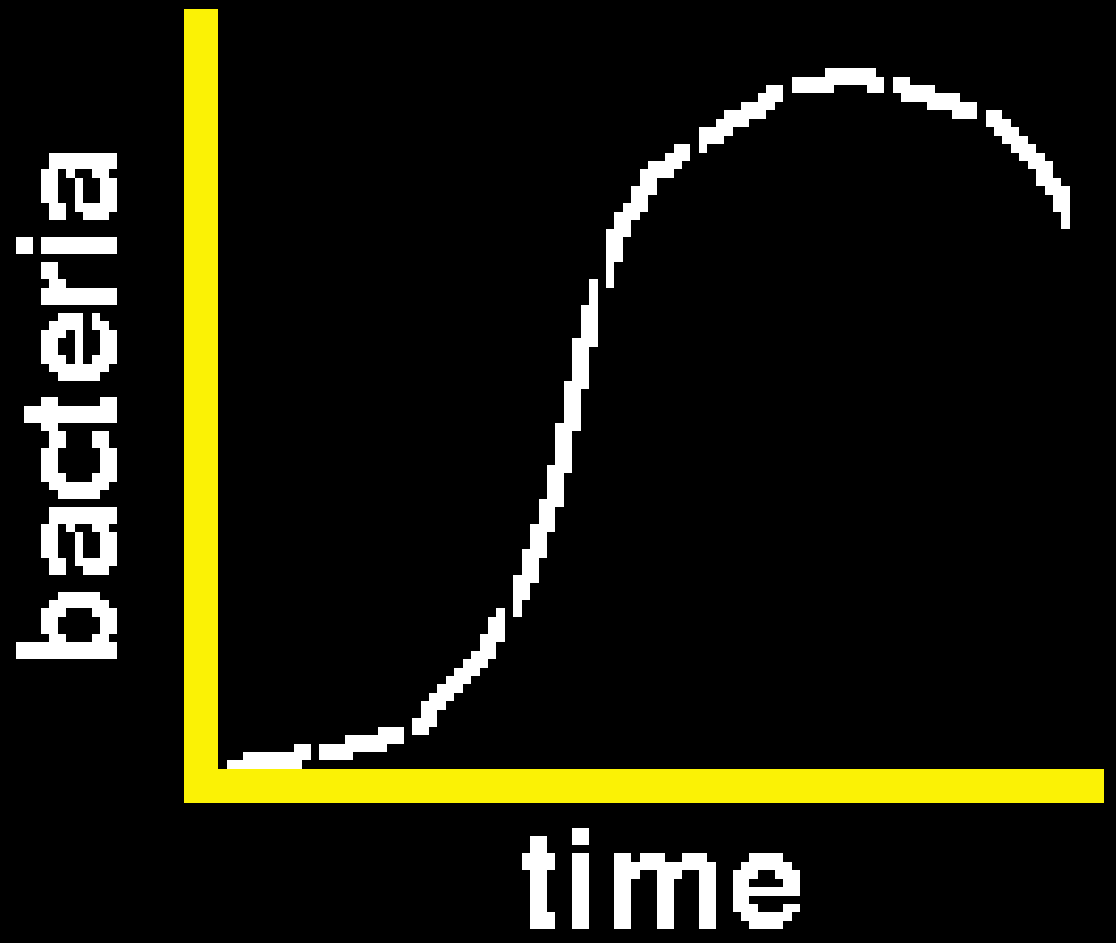

gram -ve
gram +ve
yeast
TF
Injury
Endogenous
- HSP
Exogenous
-LPS
-Lipotechoic acid
-Peptidoglycon
-Zymosan
Ligands
(PAMPS)
PRRS
NODs
TLR4
CD14
NFK B
Gene transcription
Monocyte
NO
iCAM
APC
Fibrin clot
PAI1
Fibrin
Cytokines
IL10
IL1
IL6
TNF
Thrombin
APC
TF
Neutrophil
APC
NO

Genetic Polymorphisms and Severe Sepsis

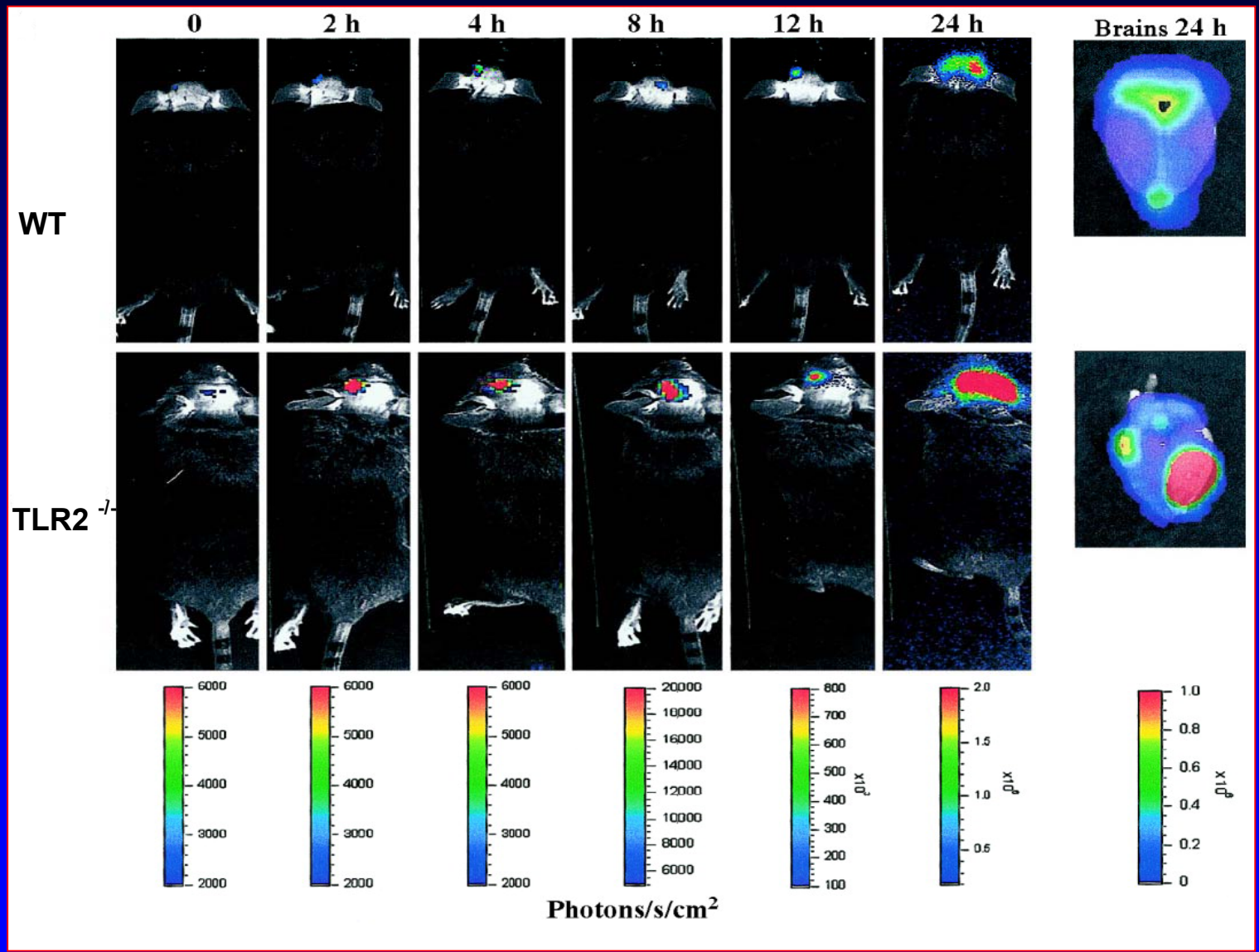
Gene	Susceptibility and/or Outcome
Mannose Binding Lectin	Meningococcemia, Pneumococcemia Severe sepsis
Toll-Like Receptor 4/2	Gram negative/positive Septic Shock
Toll-Like Receptor 5	Legionnaire's Disease
CD14	Septic Shock
FCγRII Receptor	Meningococcemia; Pneumococcemia
TNF locus	Meningococcemia Septic Shock; Cerebral Malaria
IL-18	Severe Sepsis
IL-10	Severe Sepsis, Meningococcemia
IL-6	Severe sepsis
IL-1 locus	Severe Sepsis
IL-4	Viral Pneumonia
Caspase 12	Severe Sepsis
PAI-1	Meningococcemia; Severe sepsis
FactorV Leiden	Meningococcemia; Severe sepsis

Pathogen Detection

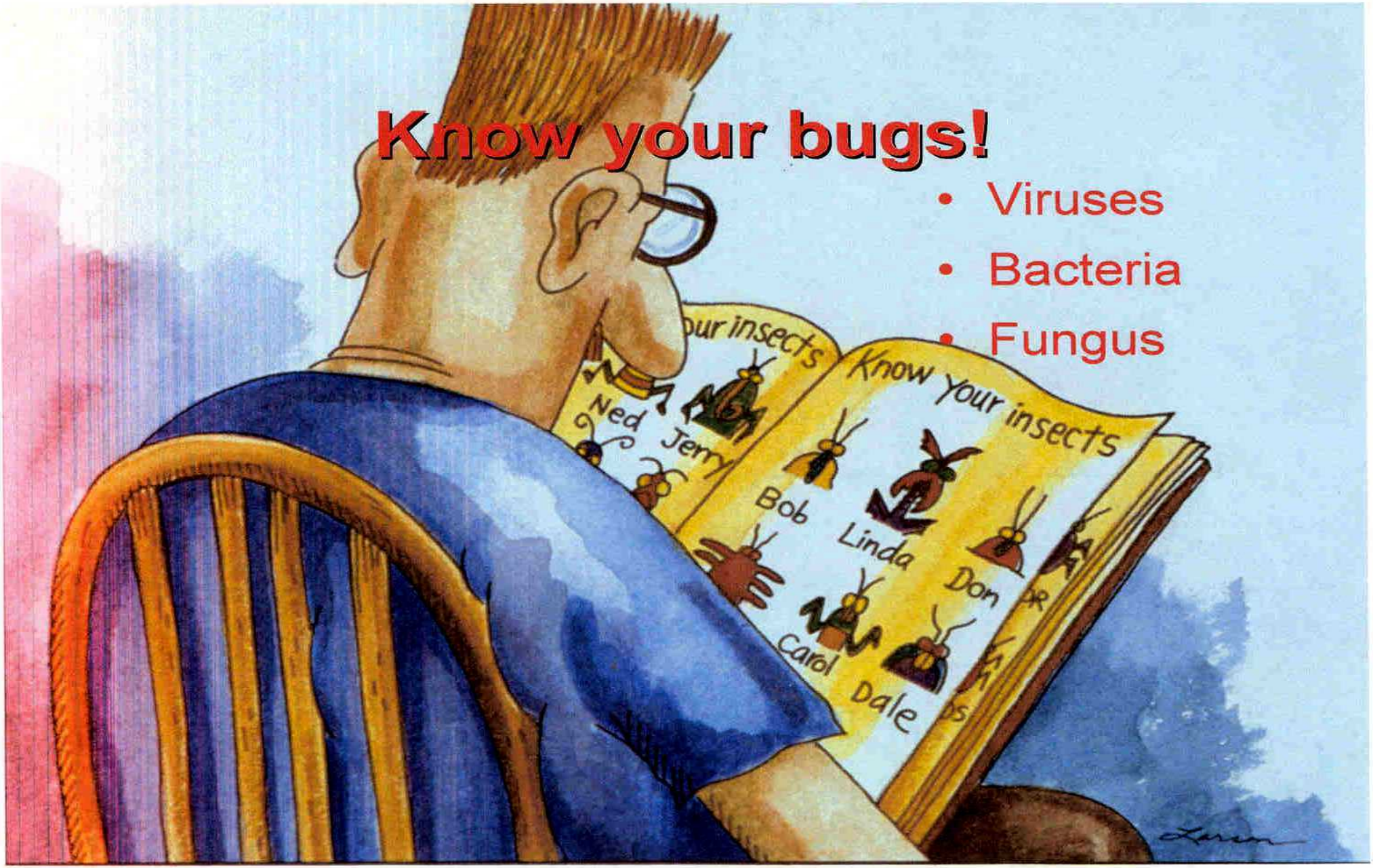


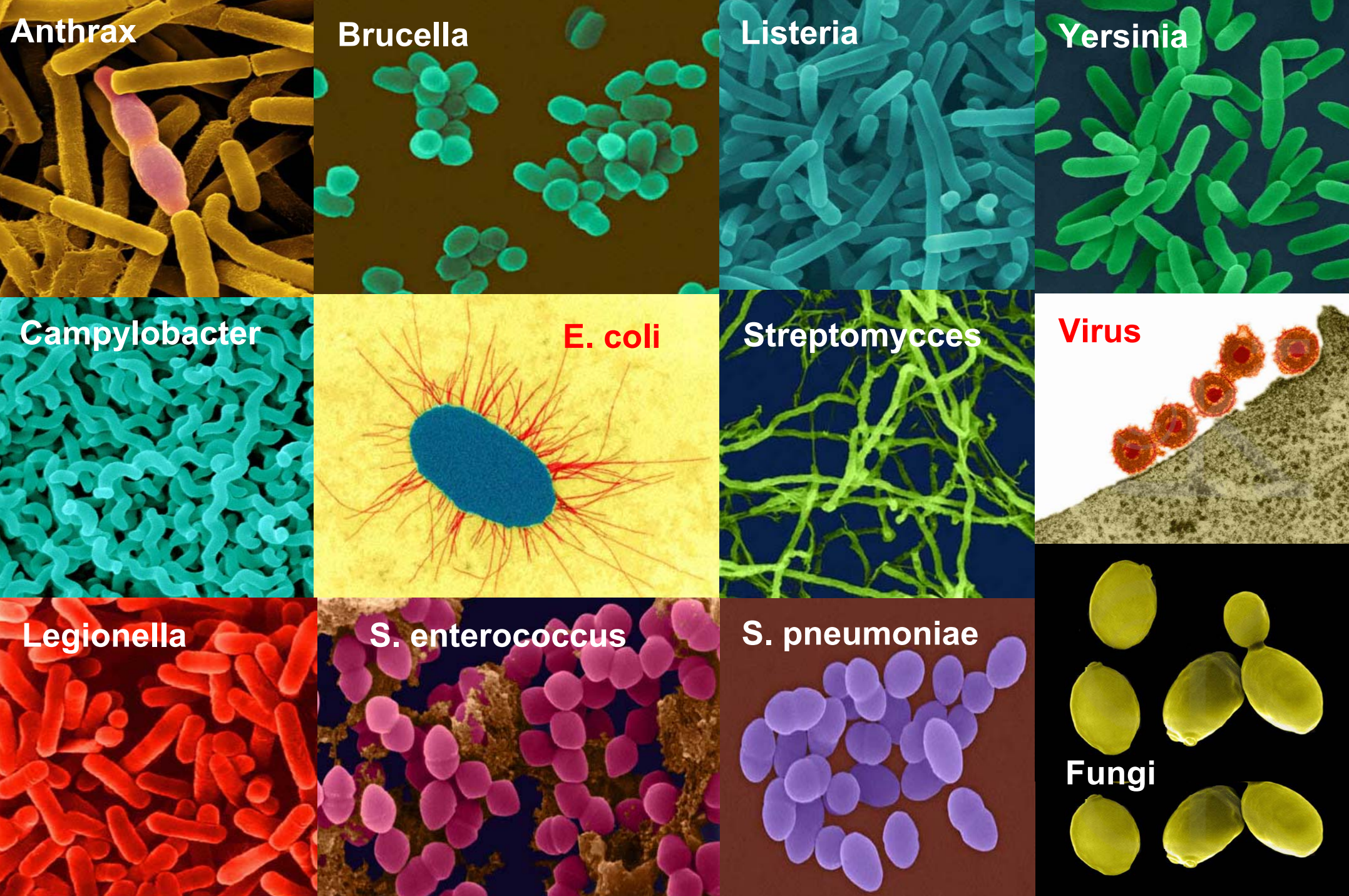

bacteria
time

TLR2 and *Streptococcus pneumoniae* meningitis

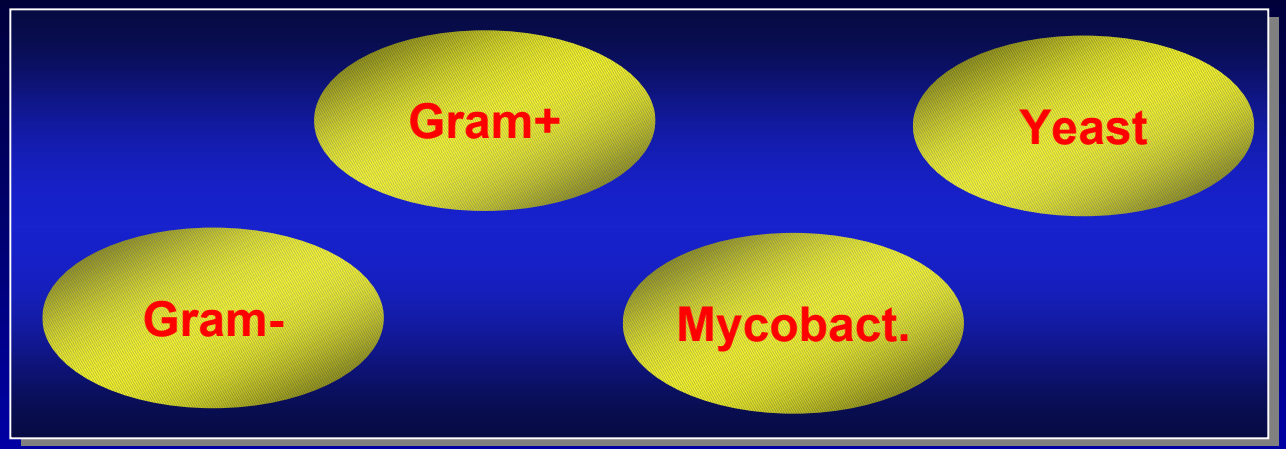


Echchannaoui H et al. JID 2002;186:798

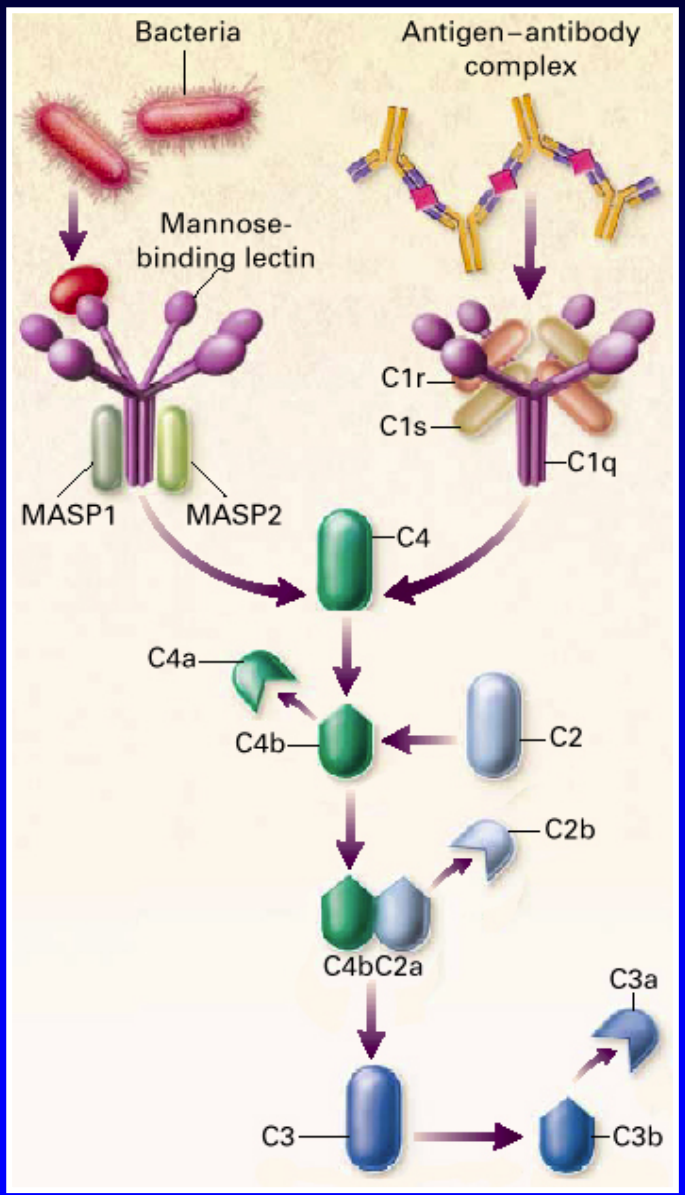

Know your bugs!
• Viruses
• Bacteria
• Fungus
Know your insects
Ned
Jerry
Bob
Linda
Don
Carol
Dale


Anthrax
Brucella
Listeria
Yersinia
Campylobacter
E. coli
Streptomycces
Virus
Legionella
S. enterococcus
S. pneumoniae
Fungi

Mannose-Binding Lectin



- Collectin
- Structural homology with C1q
- Associated to 2 serine proteases
- Variability:
  - Point mutations codons 52, 54, 57
  - Polymorphisms in the promoter



Mannose-binding Lectin Polymorphisms & The Risk of Infections

- **Repeated bacterial and fungal infections**

  Sumiya et al., Lancet 1991
  Summerfeld et al., Lancet 1995
  Garred et al., Lancet 1995
  Summerfeld et al., BMJ 1997

- **Infections after chemotherapy**

  Neth et al., Lancet 2001
  Peterslund et al., Lancet 2001

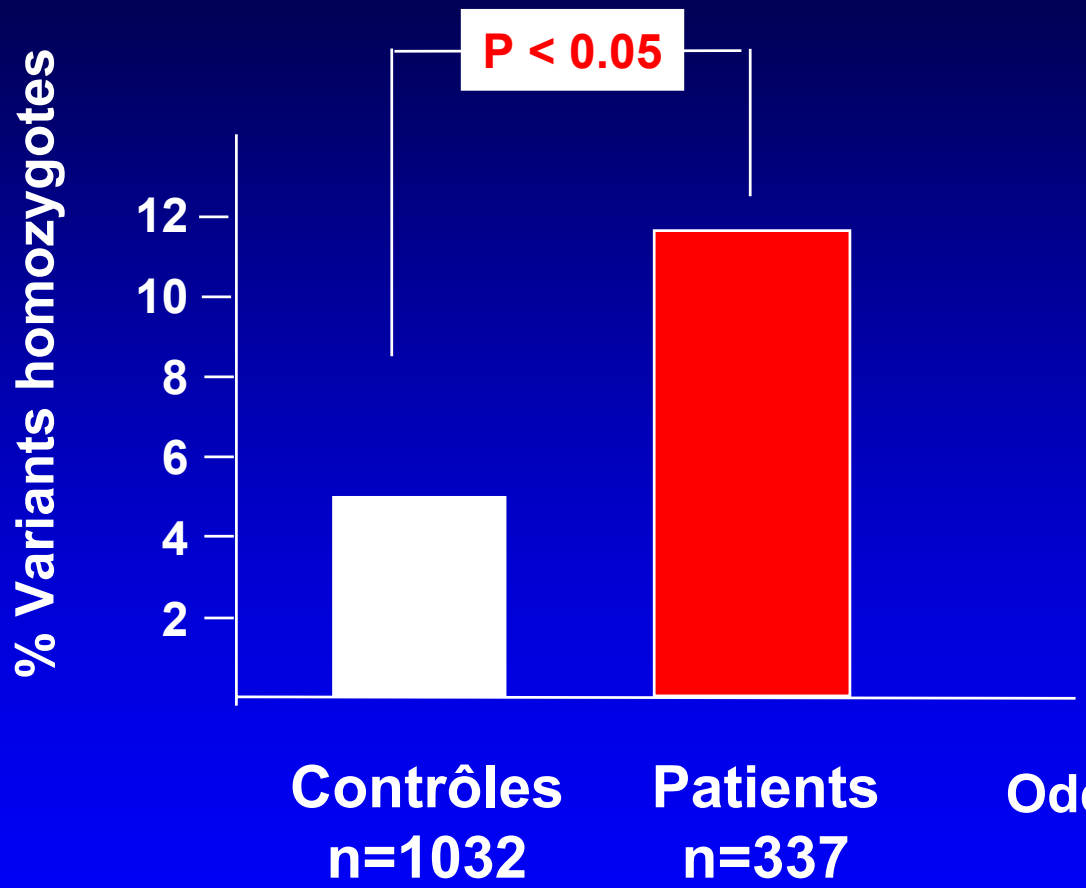
- **Increased severity of lung disease and low survival in cystic fibrosis**

  Garred et al., J. Clin. Invest. 1999

- **Meningococcal disease**

  Hibberd et al., Lancet 1999

MBL genotype and risk of invasive pneumococcal disease



Odds ratio 3.48 (1.51 – 8.01); p=0,003

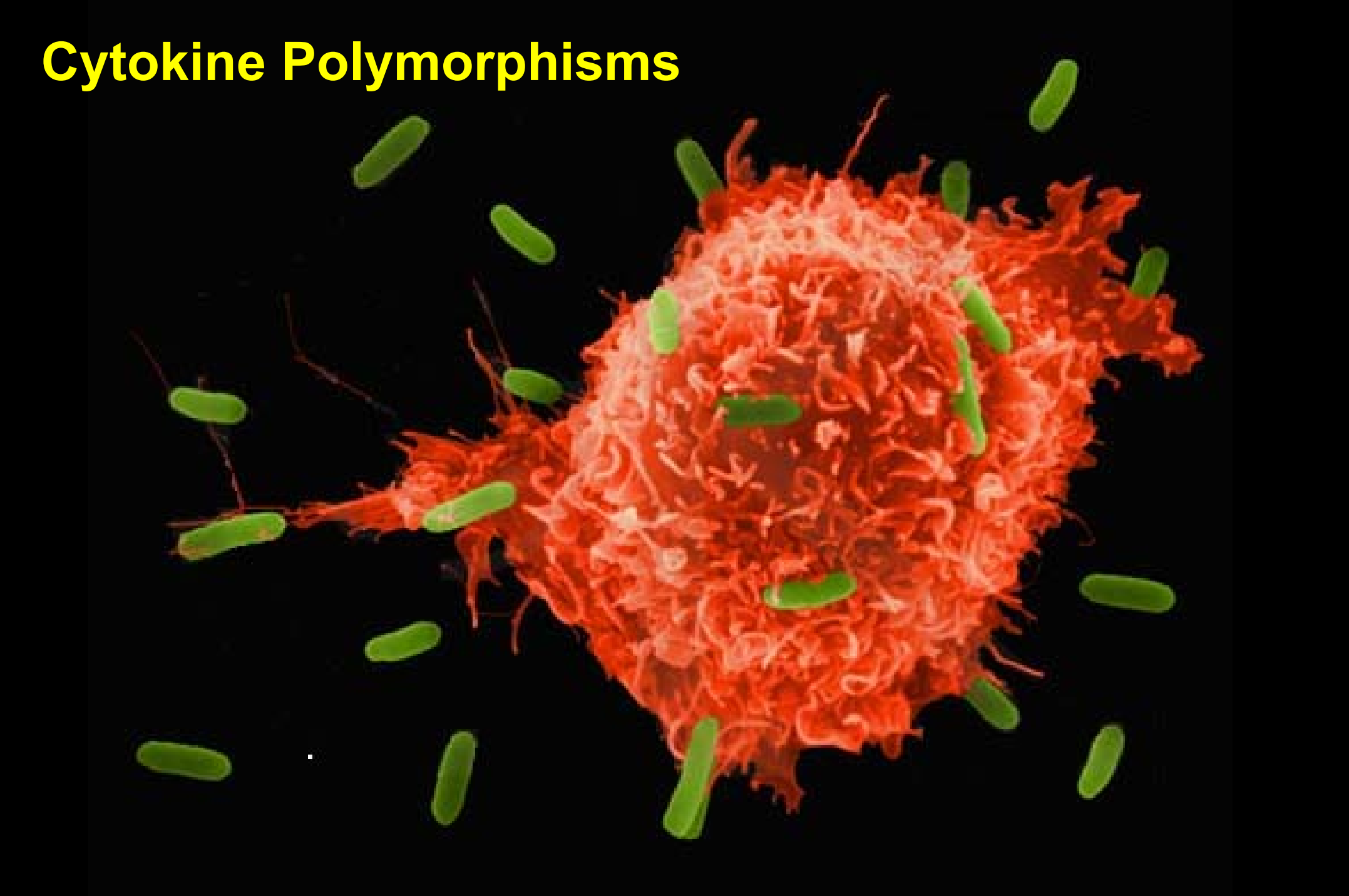
Roy et al. Lancet 2002; 359: 1569-1573

Clinical potential of mannose-binding lectin-replacement therapy

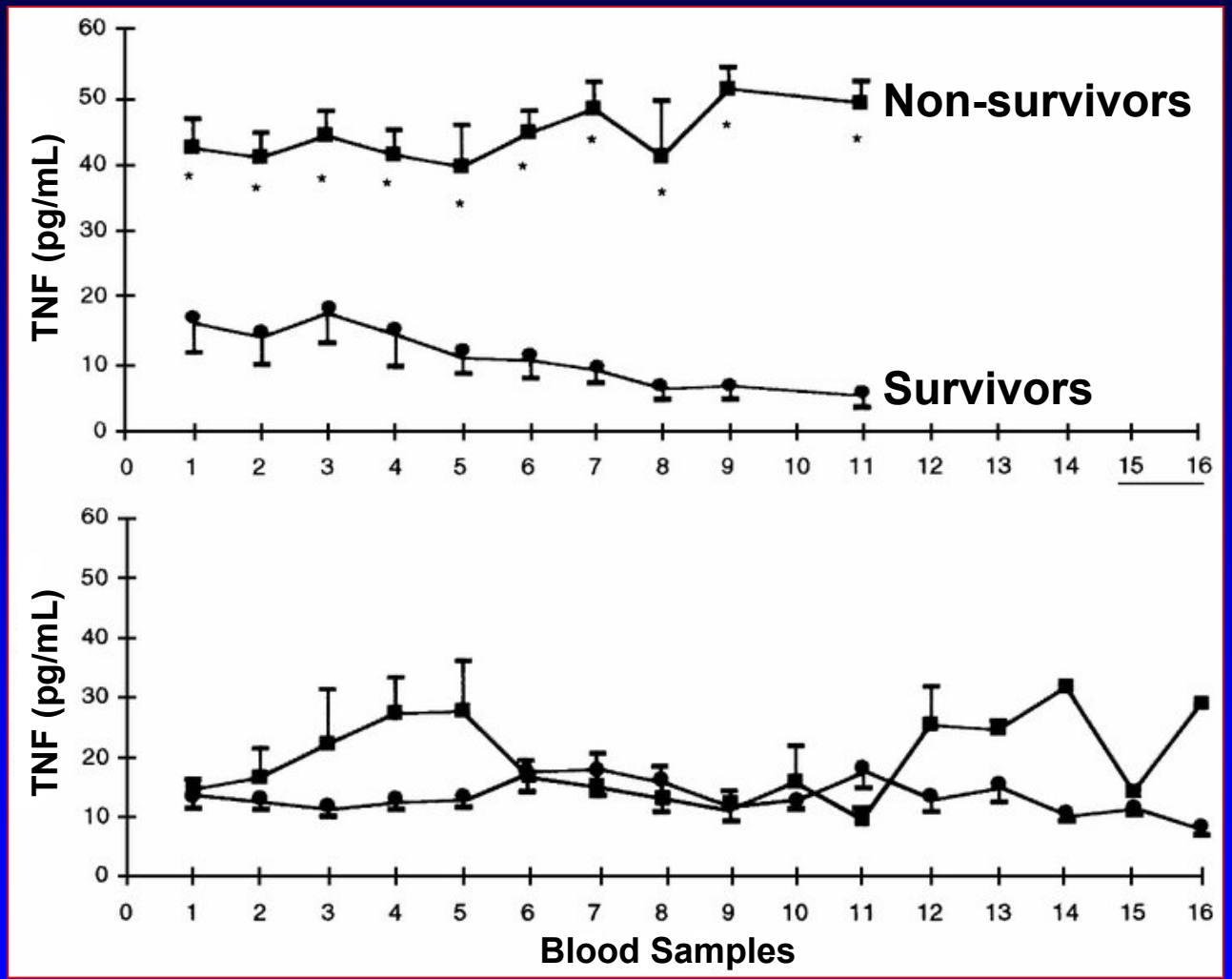
J.A. Summerfield[1]

Division of Medicine, Faculty of Medicine, Imperial College London, St Mary's Campus, London W2 1NY, U.K.

Cytokine Polymorphisms



TNF plasma levels and mortality

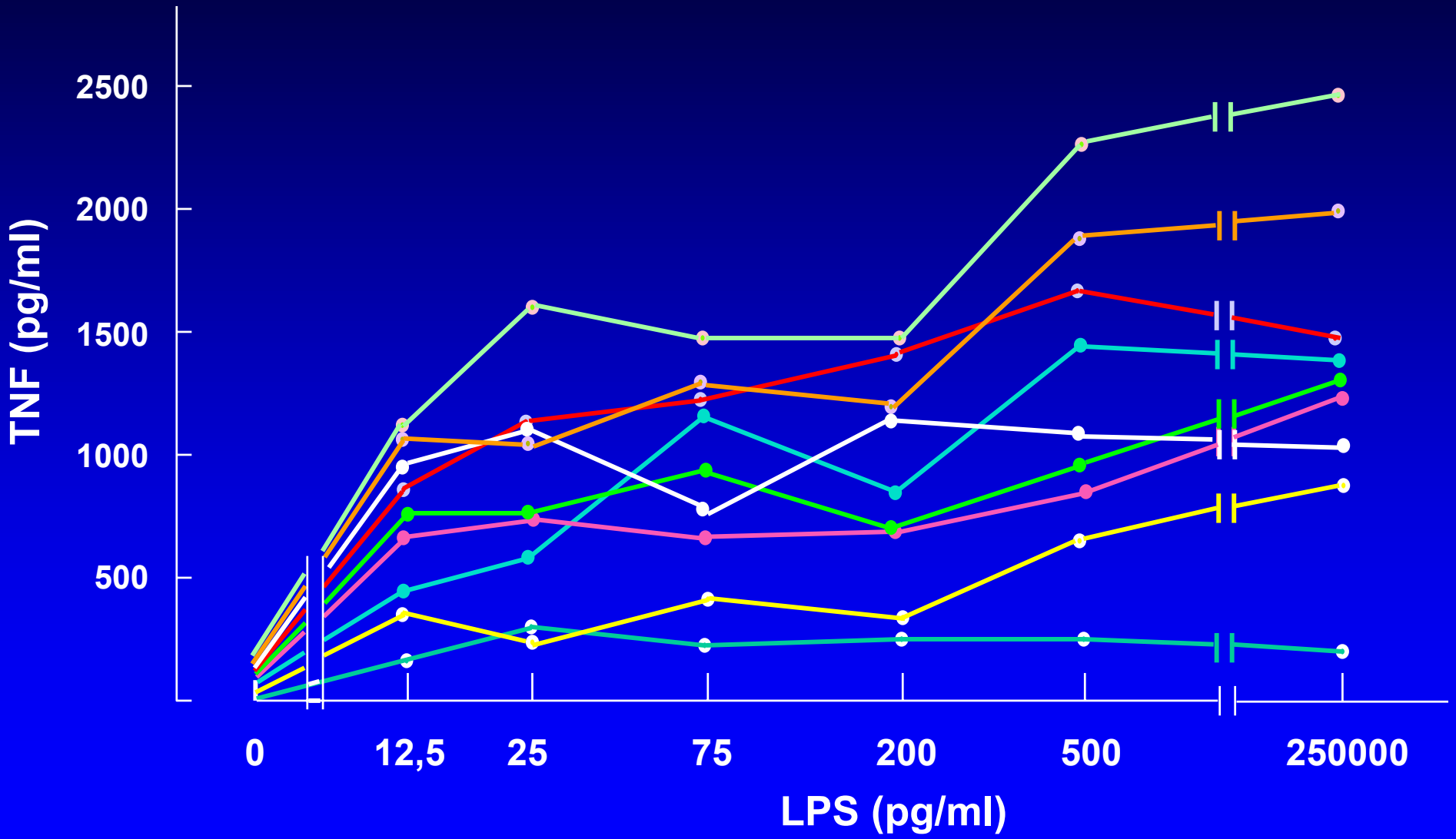


Septic shock

Trauma

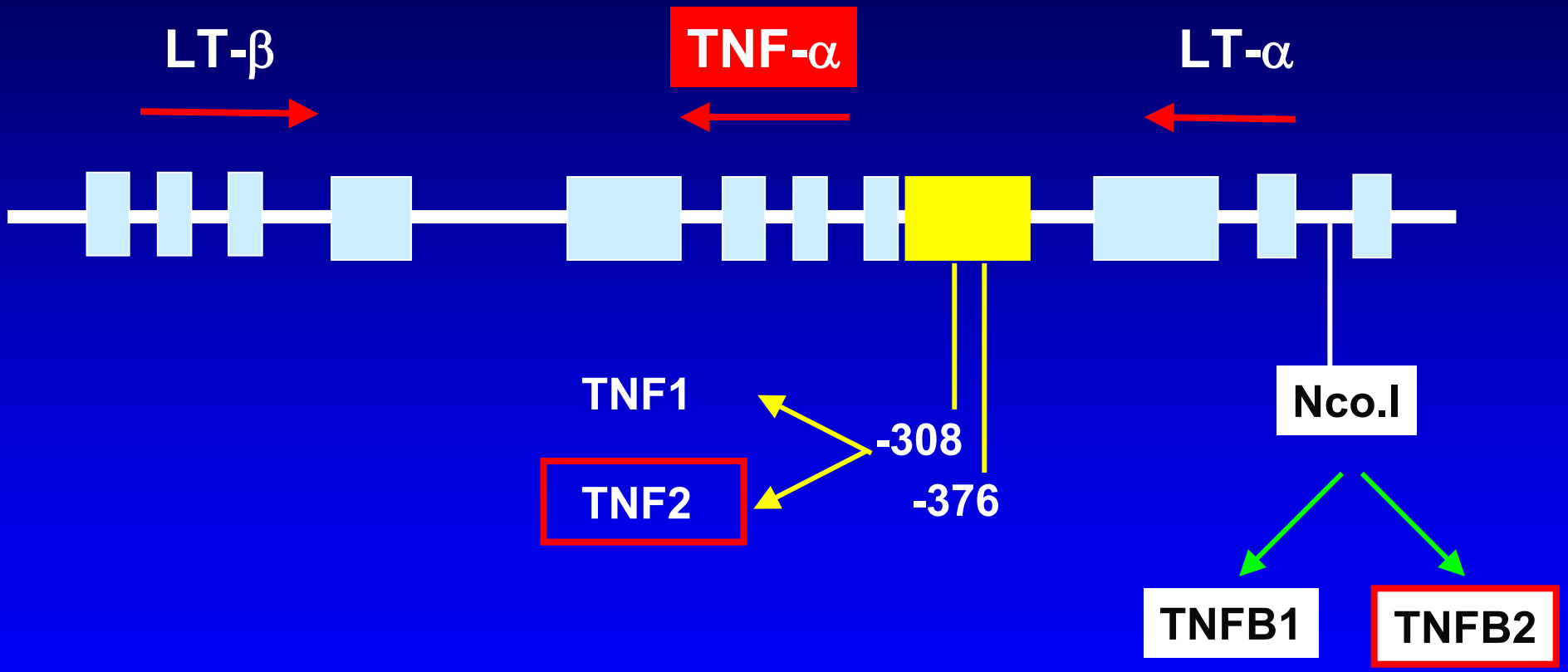
Martin C et al. Crit Care Med 1997;25:1813

Interindividual Differences in TNF-α Secretion

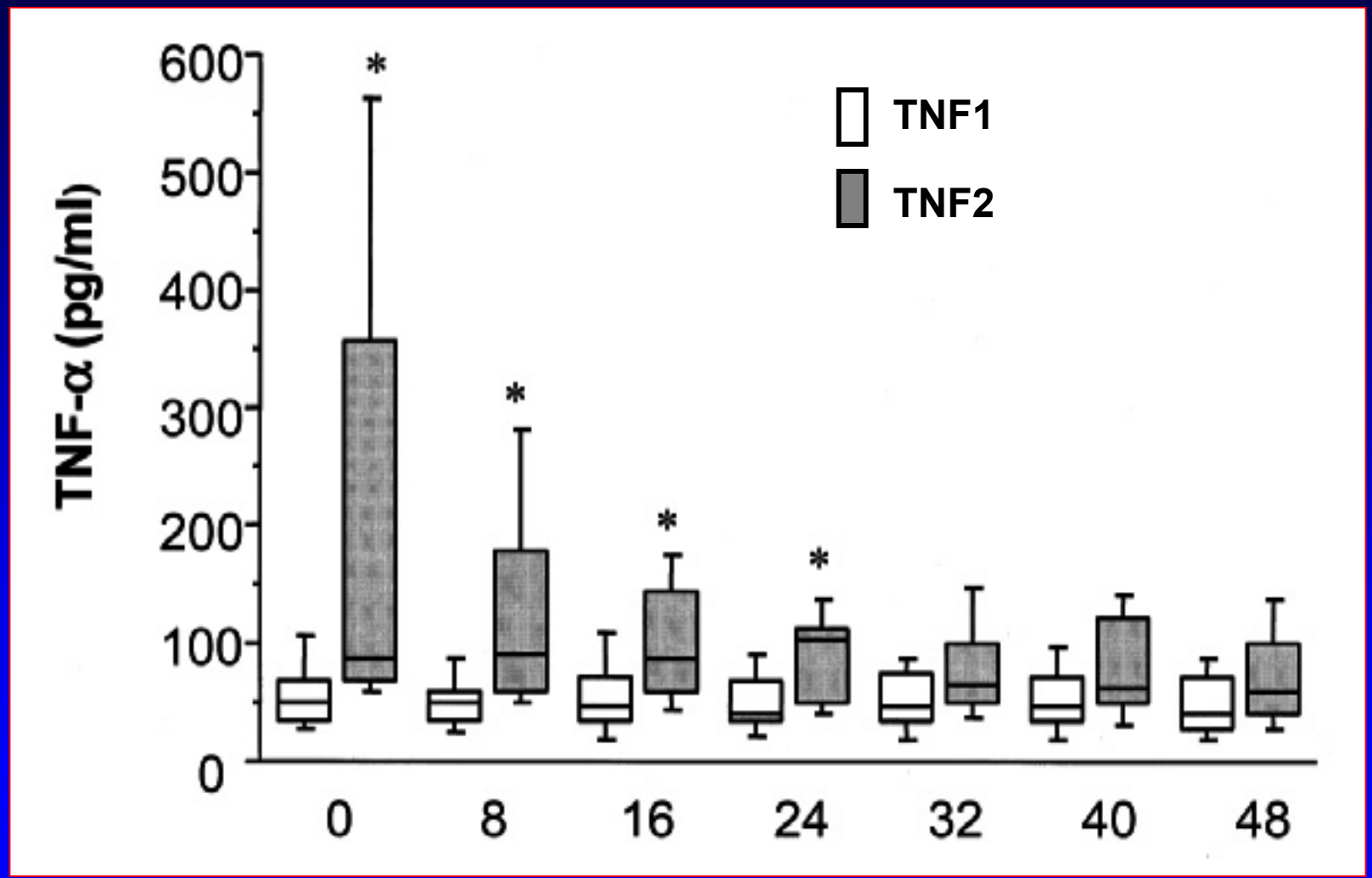


Molvig, 1988

TNF locus

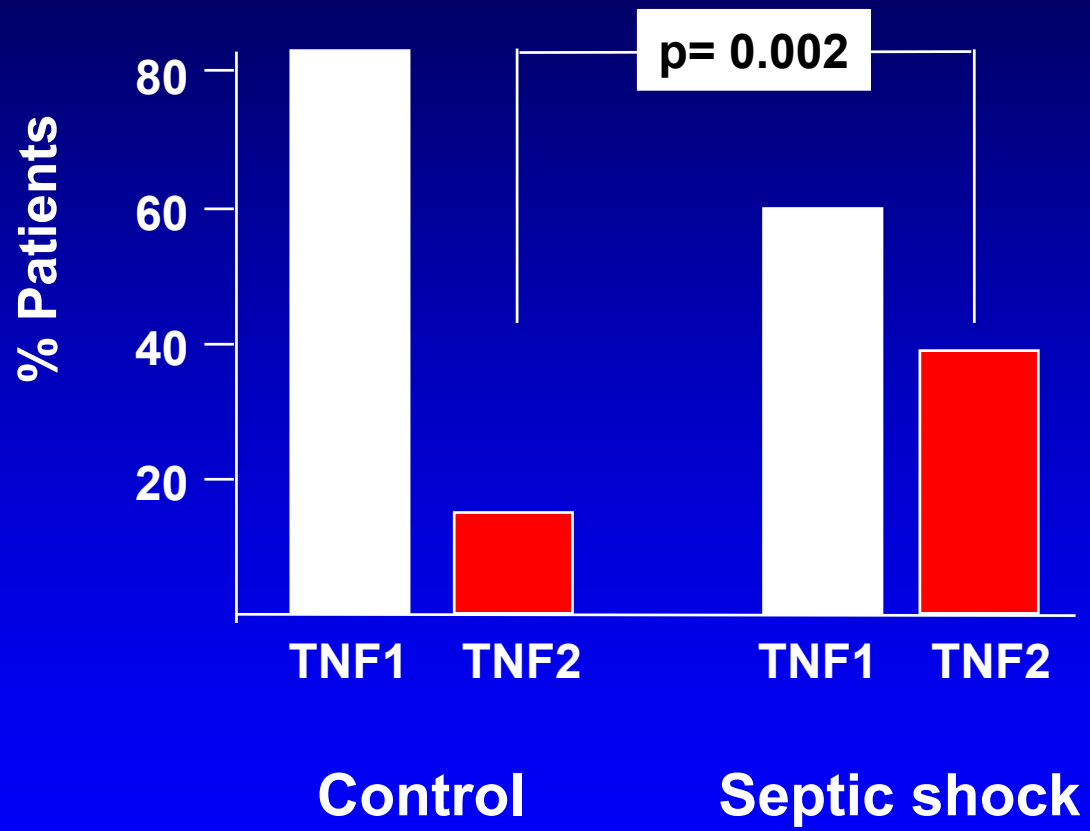


Association of TNF2 with TNF levels in Septic Shock



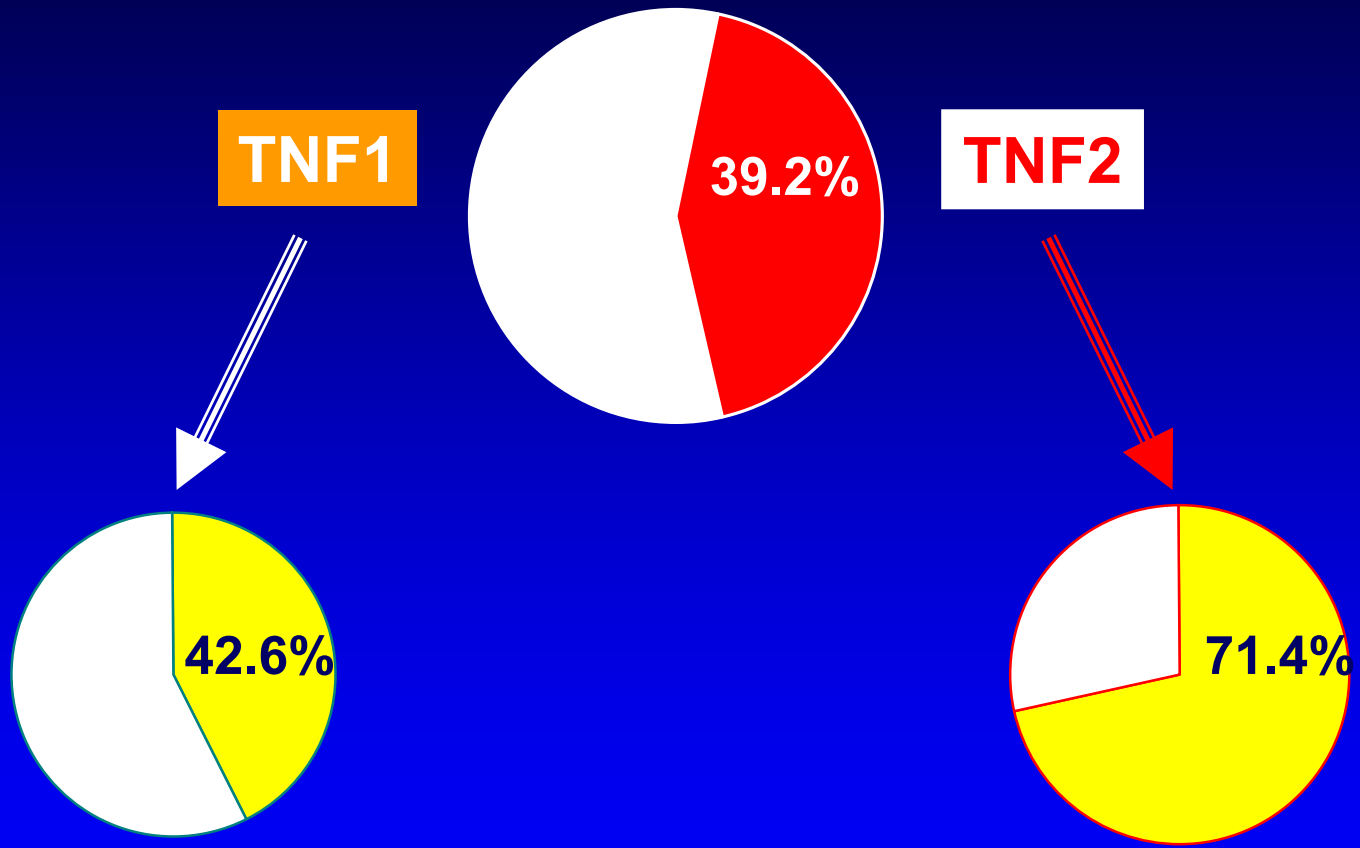
Appoloni O. Am J Med 2001; 110:486

TNF2 polymorphism and septic shock susceptibility



Mira JP. JAMA. 1999, 282:561-8

TNF2 polymorphism and septic shock outcome



Mira JP. JAMA 1999;282:561-8

TNF2 polymorphism and septic shock mortality

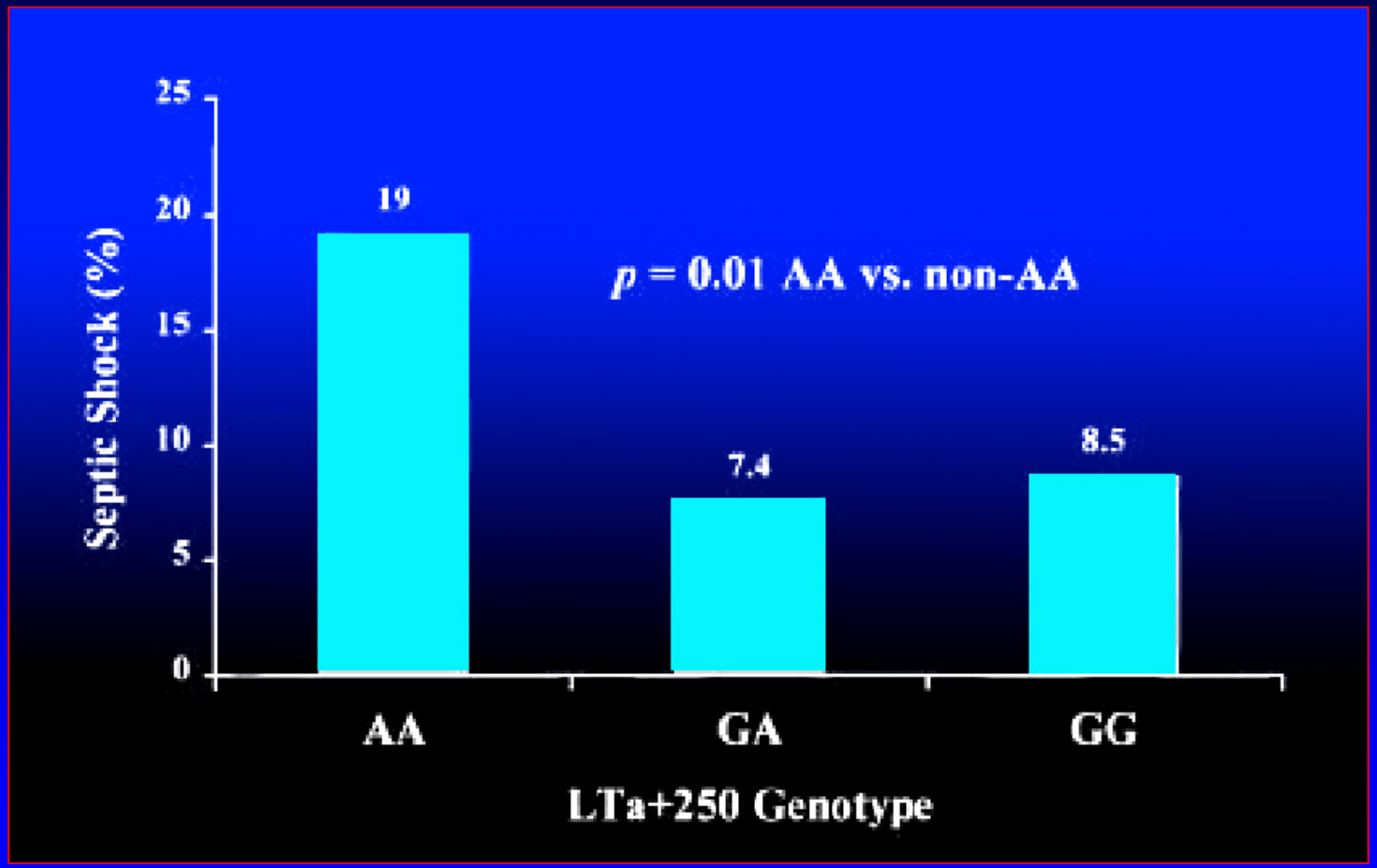
Characteristics	TNF1 (n=54)	TNF2 (n=35)	p
Age [mean. ± SD]	57 ± 15	59 ± 16	ns
SAPS II [mean. ± SD]	54 ± 17	56 ± 22	ns
OSF [mean. ± SD]	3 ± 1	2.8 ± 1	ns
Observed mortality (%)	42.6	71.4	0.008
Predicted mortality (%)	52.1	52.8	ns

Mira. JAMA 1999;282:561-8

Community-Acquired Pneumonia and TNF polymorphisms

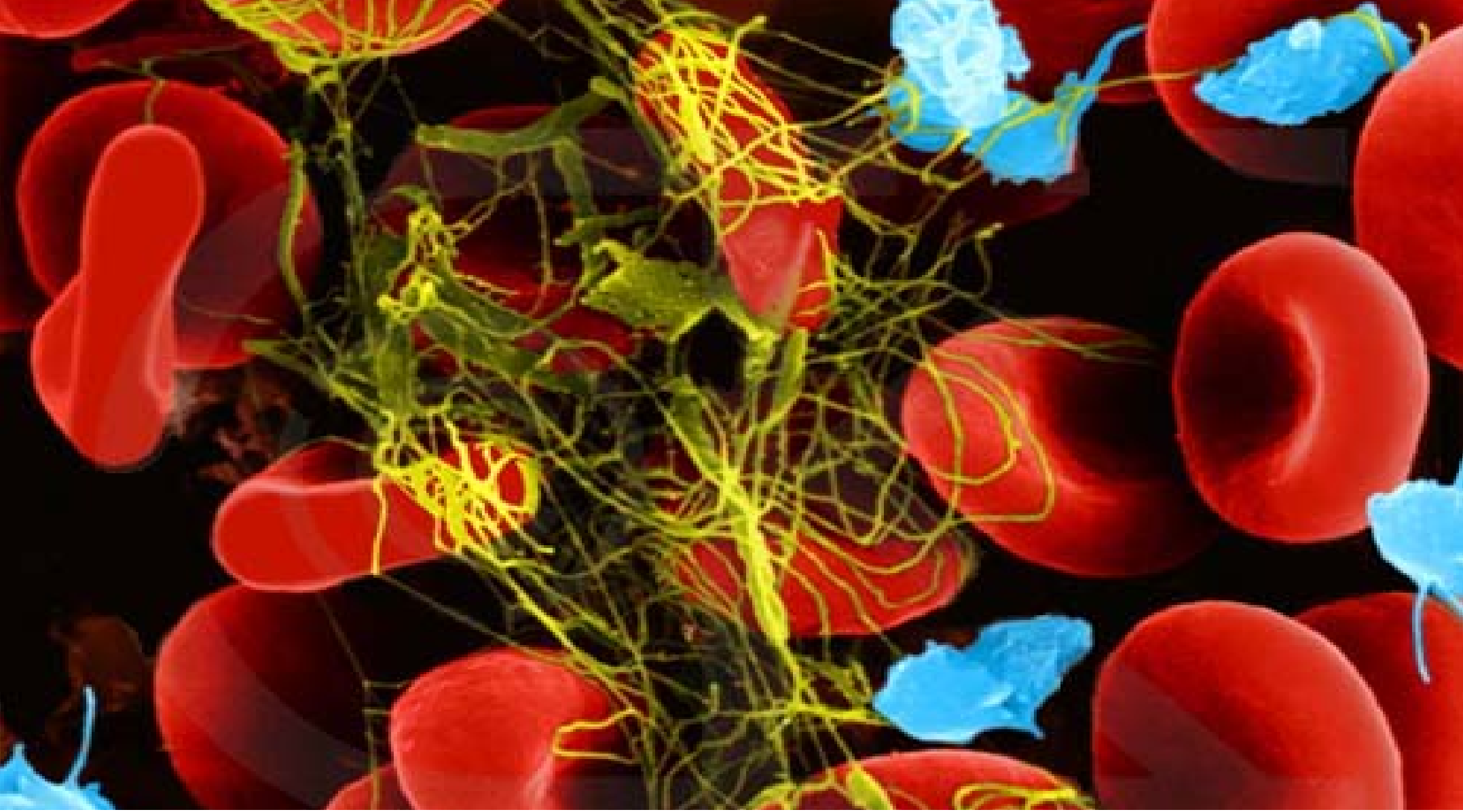
280 CAP

No association with mortality rate

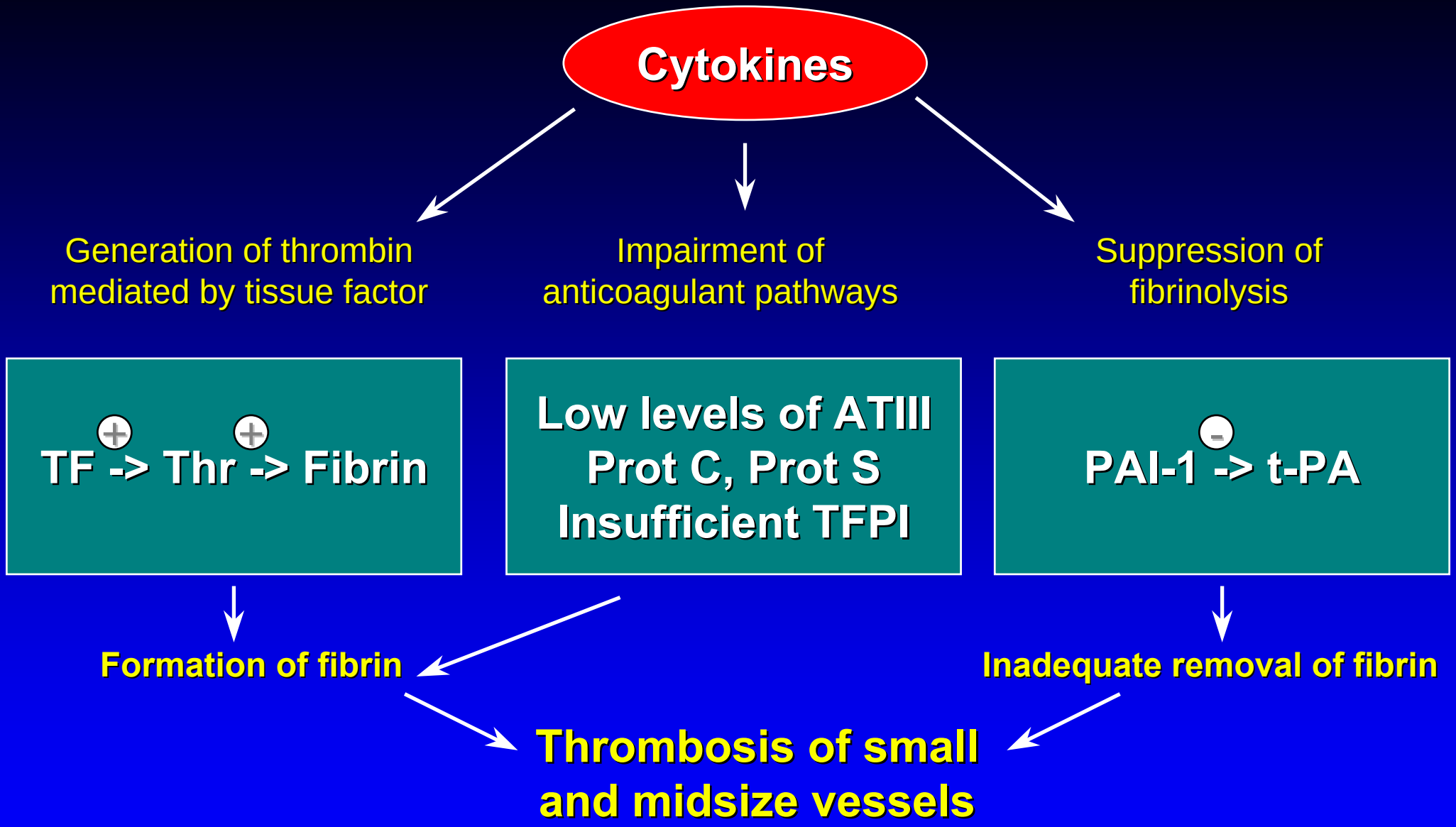


LTα+250 AA genotype RR= 2.48 (1.28 – 4.78), Age-adjusted RR = 3.64 (1.28 – 10.66)

Waterer GW. AJRCCM 2001; 163: 1599

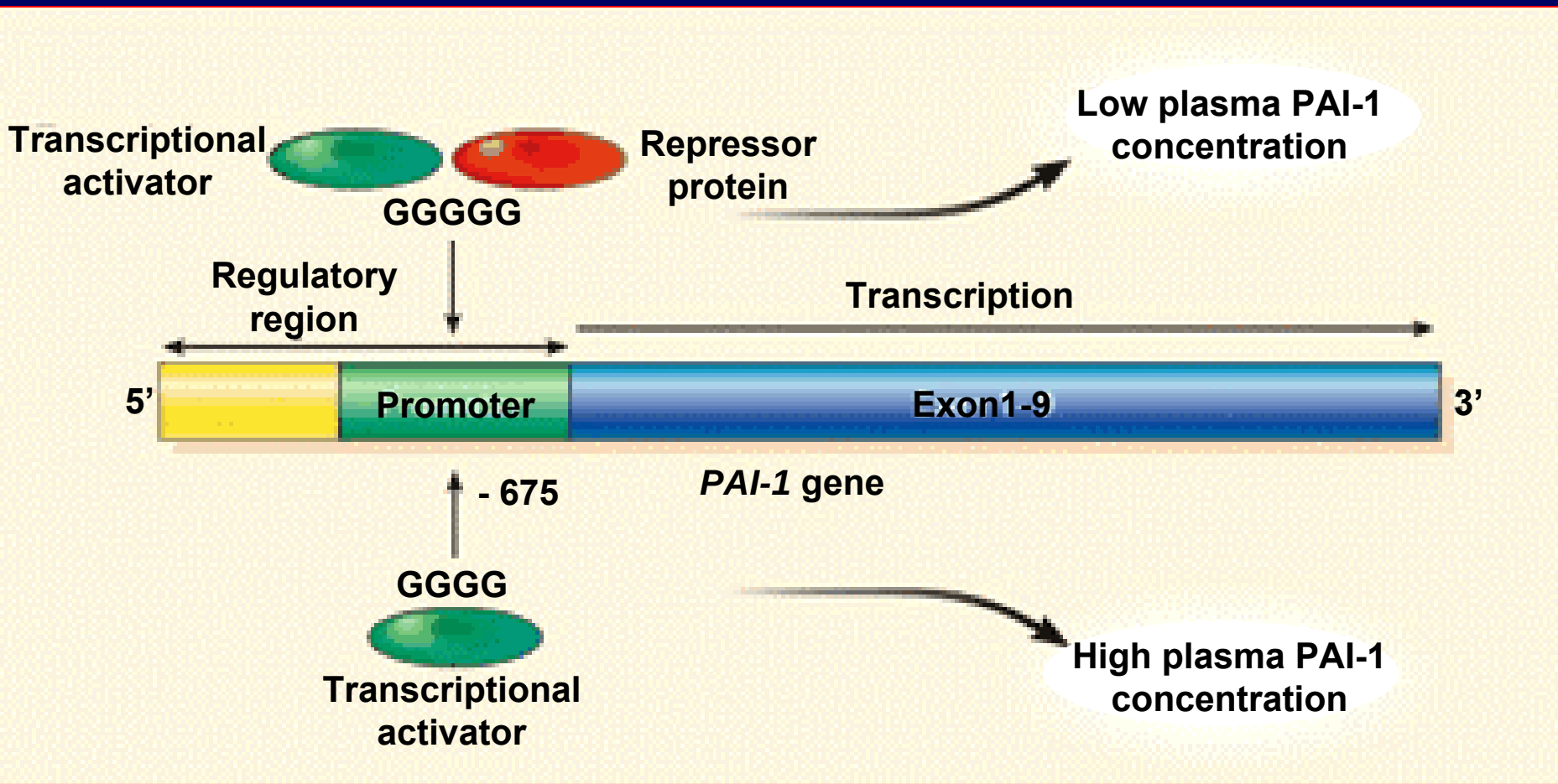


Coagulation Polymorphisms



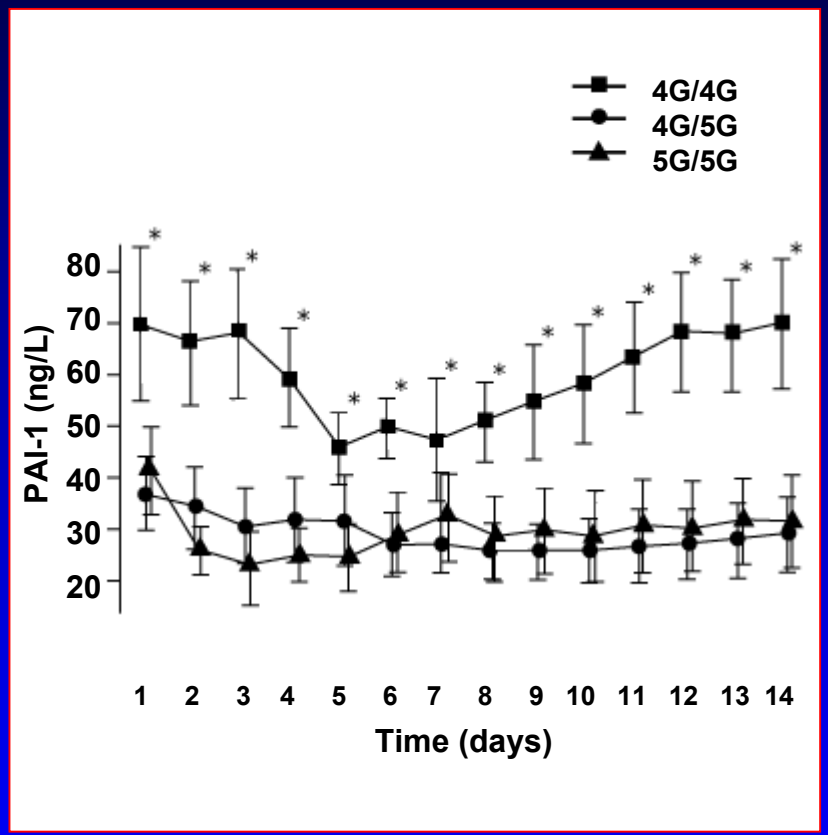
After Levi et Ten Cate, N Engl J Med 1999;341:586-92

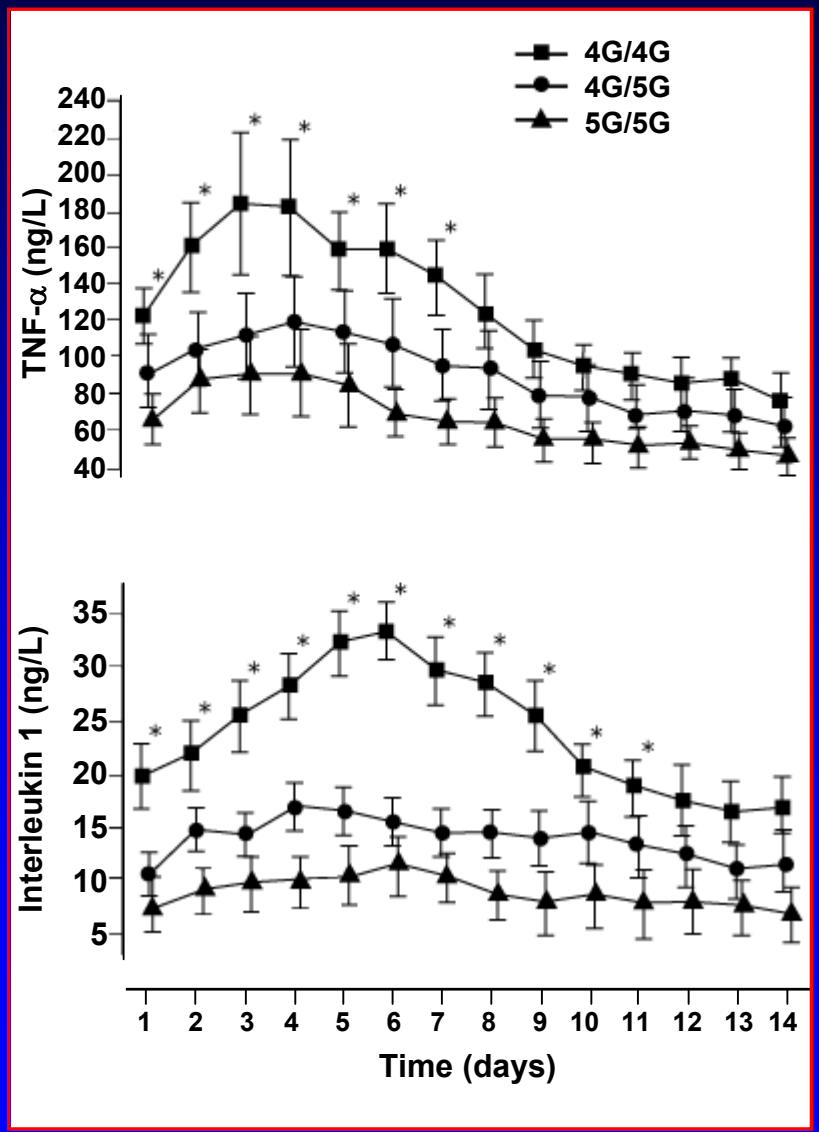
4G/5G PAI-1 Polymorphism



Genetics and Trauma Outcome

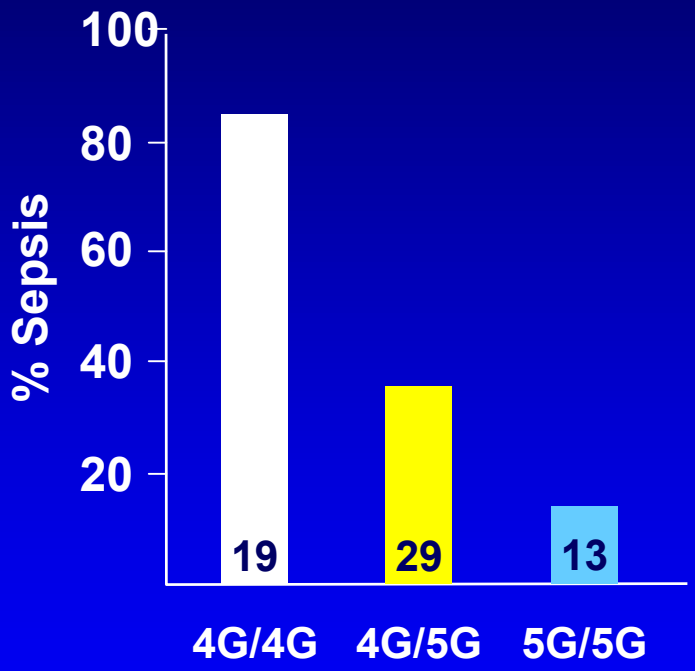
4G/5G promoter polymorphism in the PAI-1 gene and severe trauma patients

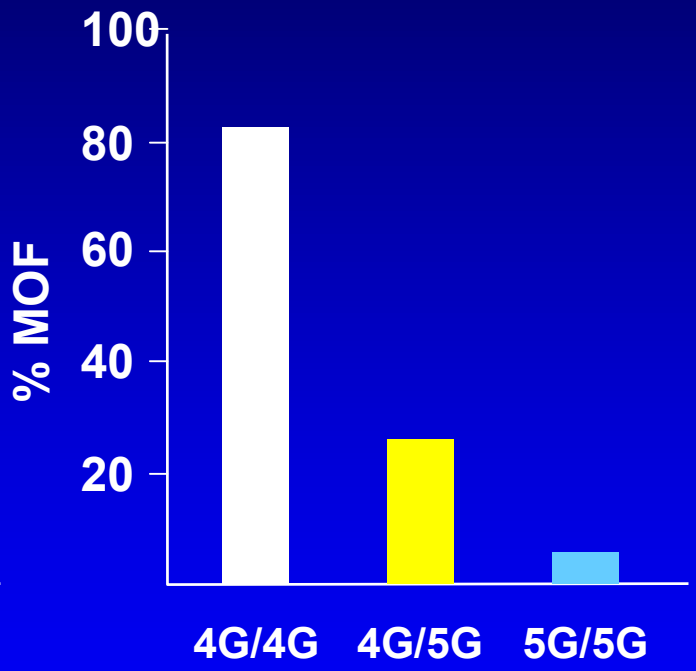


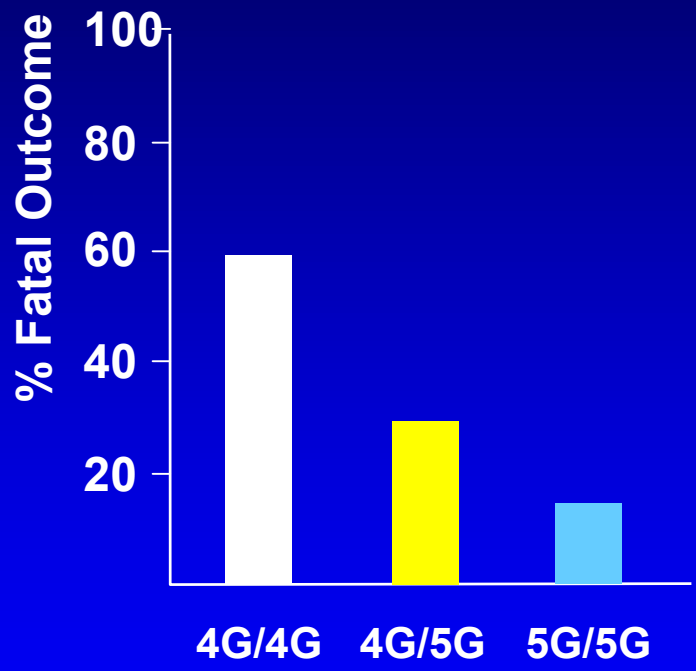


Menges, Lancet 2001;357:1096

4G/5G promoter polymorphism in the PAI-1 gene and severe trauma patients

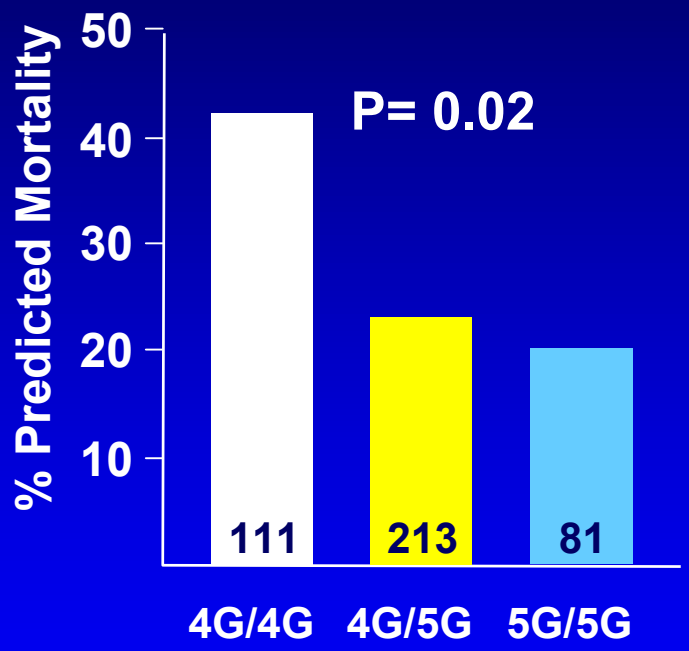


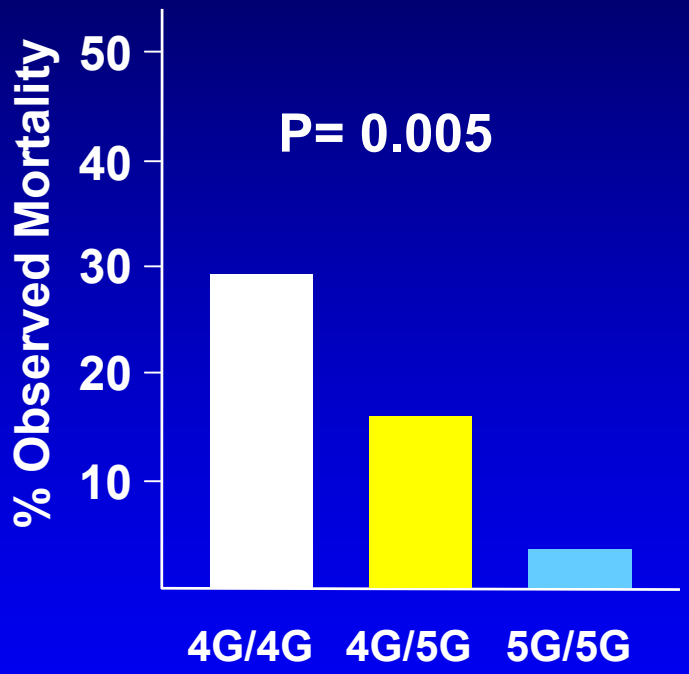


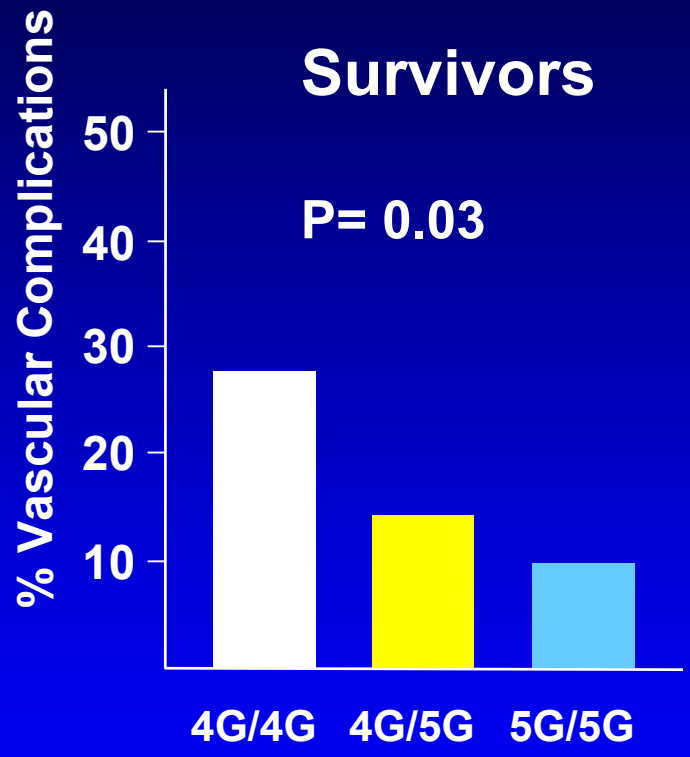


Menges, Lancet 2001;357:1096

4G/5G PAI-1 Polymorphism and Meningococcal Disease







Haralambous E. Crit Care Med 2003;31:2788

Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms in large cohorte

⇒ SNPs or haplotype

⇒ Micro-arrays, Taqman, Mass Spectroscopy, …

Yamada Y et al. *N Engl J Med 2002; 347: 1916-23.*

⇒ 2819 patients with myocardial infarction

⇒ 2242 controls

⇒ 112 polymorphisms of 71 candidate genes

PAI-1, connexin 37, stromelysin

Perspectives et Conclusions

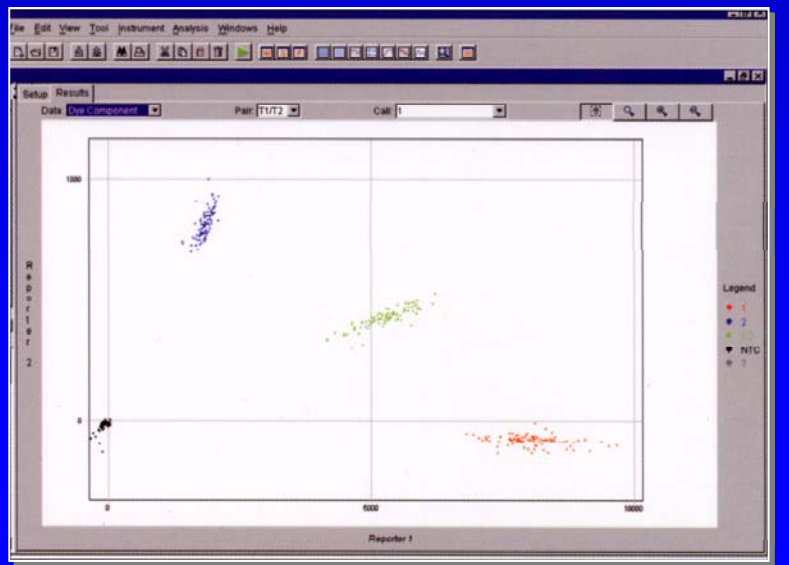
- Screening of a high number of polymorphisms in large cohorts
  - ⇒ UK: 1000 Patients – Peritonitis
  - ⇒ UK: 2000 Patients – Community-Acquired Pneumonia
  - ⇒ USA: 2000 Patients – Severe Sepsis
  - ⇒ USA: 1500 Patients – Severe Sepsis
  - ⇒ France: 3500 Patients – Nosocomial Pneumonia
  - ⇒ France: 3500 Severe Trauma
  - ⇒ Australia ?
  - ⇒ Japan ?

Génotypage à Haut Débit

20’

30’

90’



Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms

⇒ Identify potential markers of susceptibility, severity, and clinical outcome

Genetic profiling ➔ Individual risk assessment

➔ Prevention, Vaccination

➔ To tailor prescriptions to each patient

Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms

⇒ Identify potential markers of susceptibility, severity, and clinical outcome

Genetic profiling ➔ Individual risk assessment

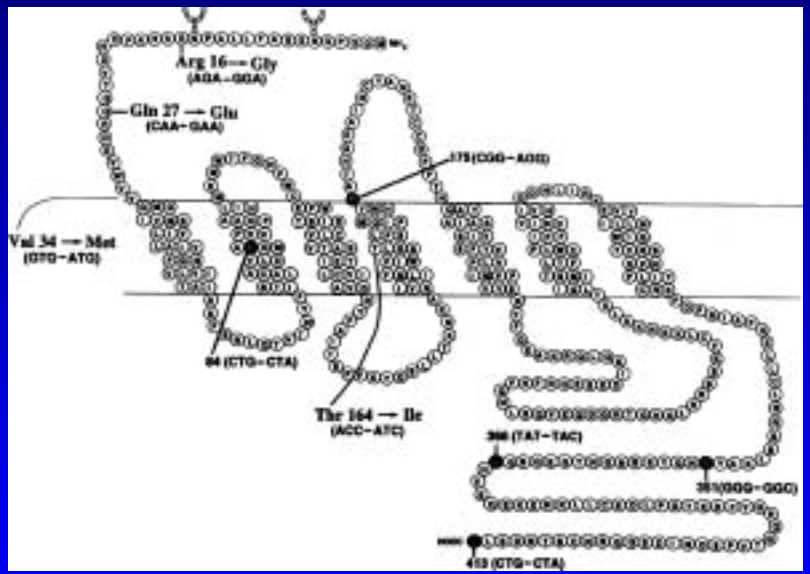
➔ To tailor prescriptions to each patient

⇒**Stratification of patients by genotype in the design of treatment trials**

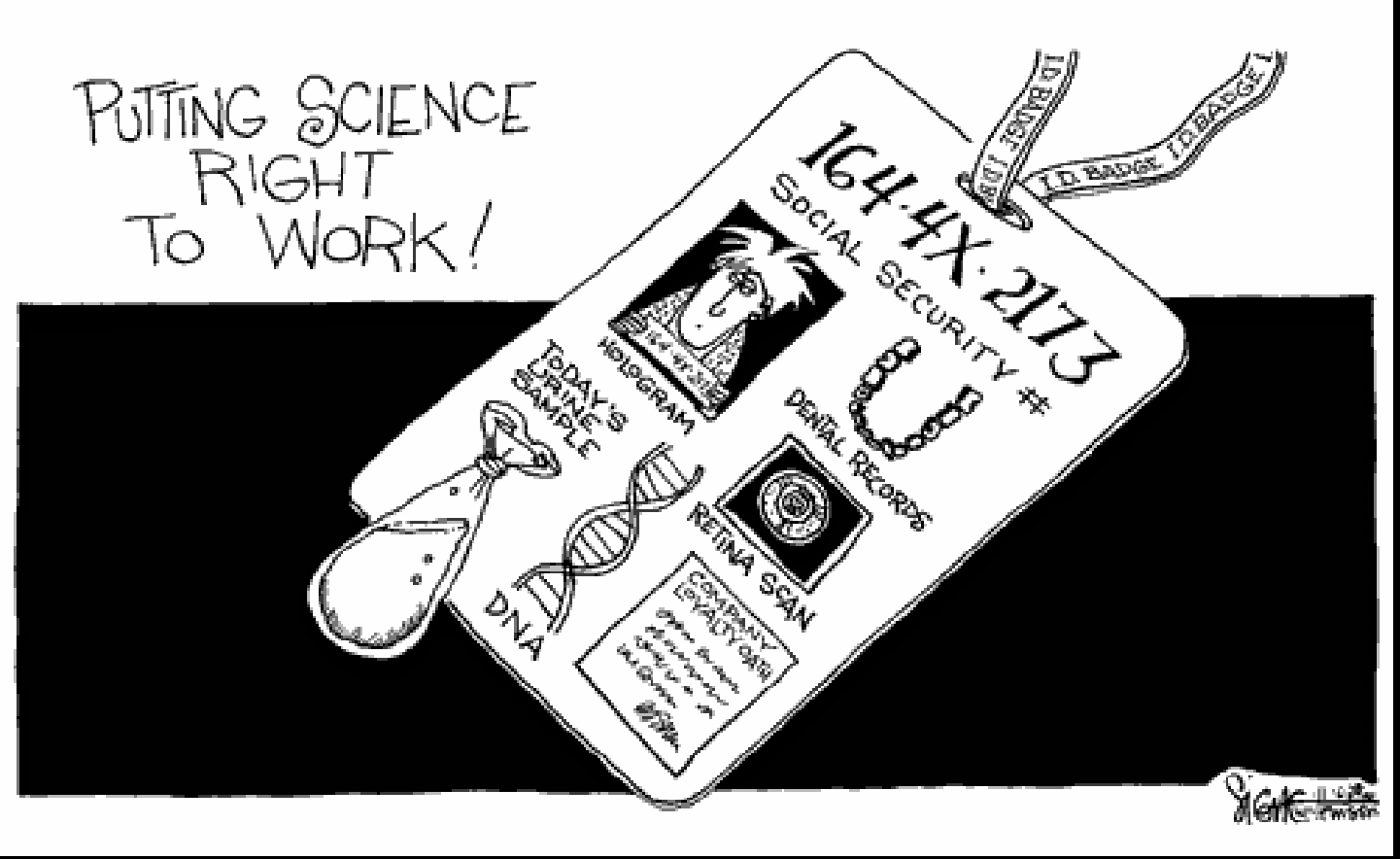
⇒**Identify potential markers for responders vs non-responders**

Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial

Elliot Israel, Vernon M Chinchilli, Jean G Ford, Homer A Boushey, Reuben Cherniack, Timothy J Craig, Aaron Deykin, Joanne K Fagan, John V Fahy, *Lancet* 2004; 364: 1505–12



Interpretation Genotype at the 16th aminoacid residue of the β_2-adrenergic receptor affects the long-term response to albuterol use. Bronchodilator treatments avoiding albuterol may be appropriate for patients with the Arg/Arg genotype.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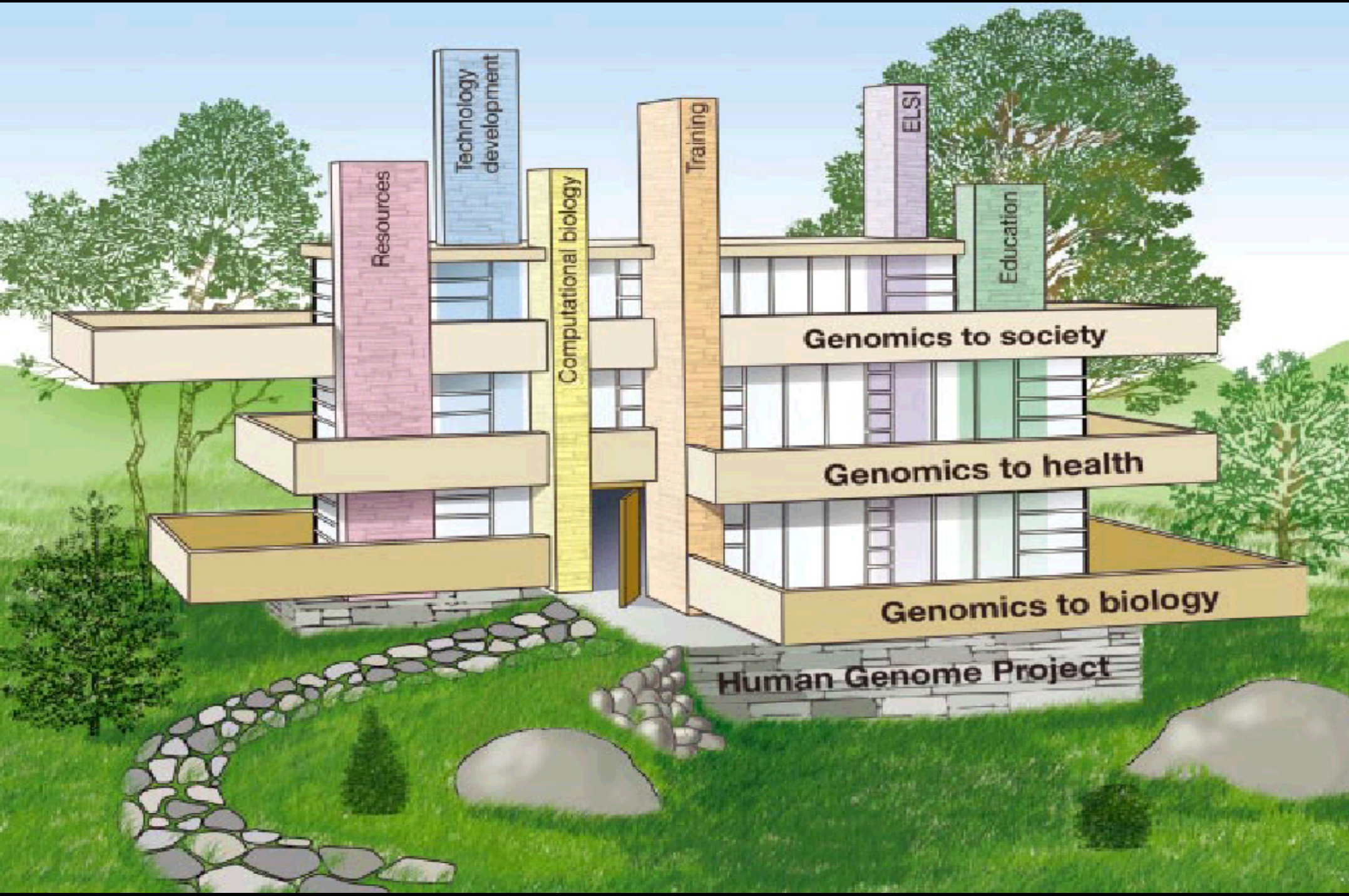
HOLOGRAM
TODAY'S URINE SAMPLE
DENTAL RECORDS
RETINA SCAN
DNA
COMPANY LOYALTY OATH


Technology development
Resources
Computational biology
Training
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Education
Genomics to society
Genomics to health
Genomics to biology
Human Genome Project

24 October 2003

Science

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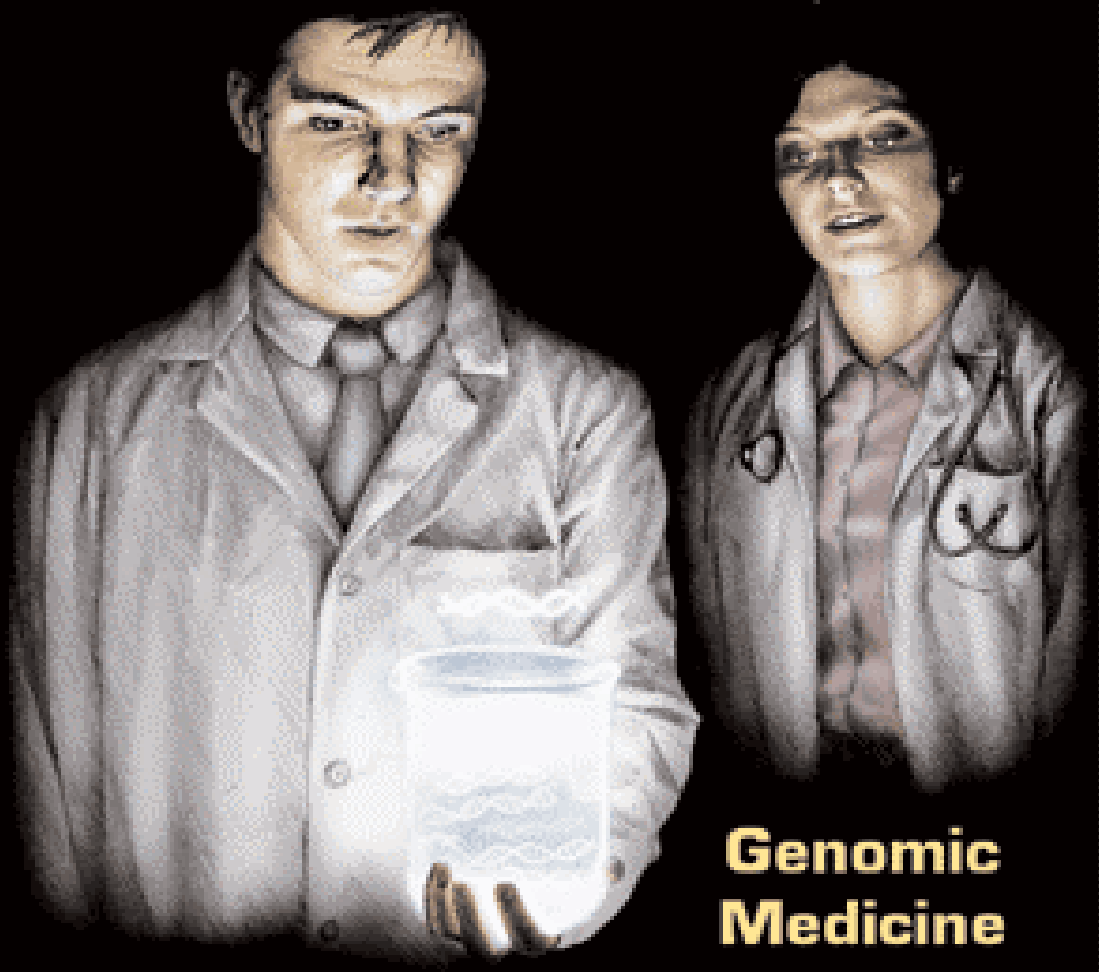

Genomic Medicine

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