KKU International Teaching Platform

UNIVERSITE PIERRE & MARIE CURIE

Khon Kaen University

Innate Immunity

1) Definition
2) Actors of innate immunity
3) Concept of PAMPs and PRRs

Jean-Marc Cavaillon
Unit Cytokines & Inflammation
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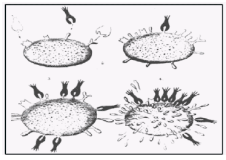
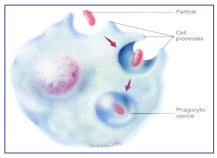
INSTITUT PASTEUR

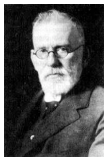
HUMORAL IMMUNITY



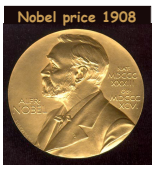
CELLULAR IMMUNITY



Paul Ehrlich



Nobel price 1908



Elie Metchnikoff

INNATE IMMUNITY

immediate

ADAPTATIVE IMMUNITY

Requires time to inform the immune system

Induces immune memory

INDUCED BY VACCINATION

INNATE IMMUNITY

INFECTION → EFFECTOR CELLS RECRUITMENT → RECOGNITION ACTIVATION OF EFFECTOR CELLS → ELIMINATION OF INFECTIOUS AGENT

ADAPTATIVE IMMUNITY

INFECTION → CONVEYING OF ANTIGEN TOWARDS LYMPHOIDS ORGANS → RECOGNITION BY NAIVE B & T LYMPHOCYTES → CLONAL EXPANSION & DIFFERENTIATION IN EFFECTOR CELLS → ELIMINATION OF INFECTIOUS AGENT

PROTECTIVE IMMUNITY & IMMUNOLOGICAL MEMORY

RE-INFECTION → RECOGNITION BY PRE-FORMED ANTIBODIES AND EFFECTOR T LYMPHOCYTES → ELIMINATION OF INFECTIOUS AGENT

RE-INFECTION → RECOGNITION BY MEMORY T & B LYMPHOCYTES → FAST EXPANSION & DIFFERENTIATION → ELIMINATION OF INFECTIOUS AGENT

~~Immune system discriminates between self and non-self~~

An innate sense of Danger

Polly Matzinger offered a new definition of immunology :

The immune system does not discriminate between self and non-self, but recognizes and responds to danger signals

(*Annu. Rev. Immun. 1994*)

She postulated that any stress undergone by healthy tissues generate danger signals, which initiate the activation of the immune response.

She proposed the concept of danger as an internal signal, when pathogens are recognized by tissue dommages they generate.

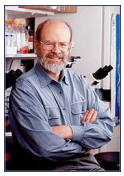
"the 4 Ds of the danger model : distress, damage, destruction, and death"

Charles Janeway

(1943–2003)

"Why do we need to add adjuvant (noxious substances like mineral oil, mycobacteria, or aluminum hydroxide) in order to get a decent response to a vaccine?" The self-nonself model neither predicted nor explained the need for adjuvant.

Professor (Yale University, New Haven, Connecticut)



In 1989, Charles Janeway proposed that the self-non-self model had reached its end, and argued that the innate immune system was the real gatekeeper. He also argued that the innate immune system used ancient pattern-recognition receptors to recognize a pathogen by its unchanging characteristics.

Natural barriers : Skin, epithelium mucosa, mucus

Complement system

Natural anti-bacterial agents : Enzymes, surfactant, defensins…

The actors of innate immunity

Phagocytic cells

Natural killer cells

cytokines

E. Metchnikoff

(1845-1916)

Discovery of phagocytosis

1867 – Thesis on embryogenesis of arthropods (Saint-Petersburg University).

1869-1873 – professor, Saint-Petersburg University

1870 – Zoology professor, Odessa University

April 1873 – Death of his 1st wife, Ludmila Vassilievna Fedorovna (Tuberculosis). Desperate, he attempts to kill himself, swallowing a large dose of opium.

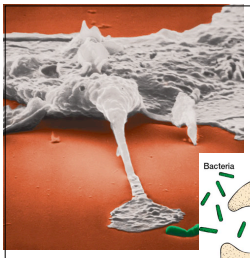
1874 – He married Olga Belokopitova. After she had a typhoid fever (1880), again he attempted to kill himself !

1882 – He traveled to Messina (Italy) with his wife to work on comparative embryology, and discovered phagocytosis.

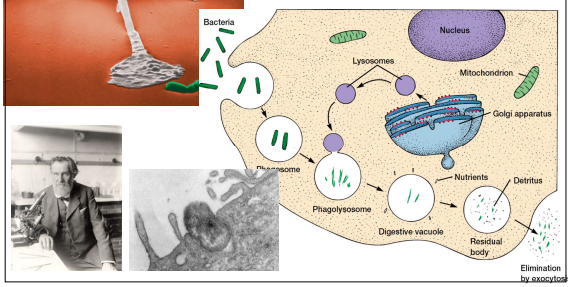
1885 – Director of the new Bacteriology Institute in Odessa (after N. Gamaleïa, back from Paris, brought back the ways to treat rabies)

Oct. 1888 – 1916 – Offered to join the new Institut Pasteur, he become head of the Unit "Morphological Microbiology". He worked on phagocytosis, immune system, ageing and intestinal flora.

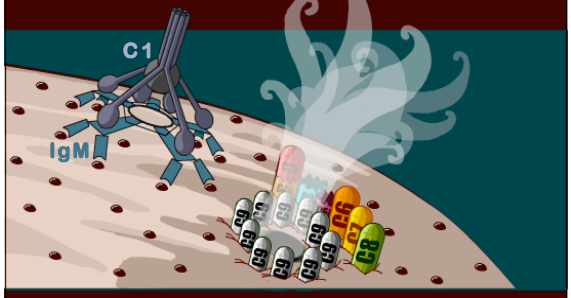
1904 – 1916 – deputy-director of Institut Pasteur



Discovery of phagocytosis



COMPLEMENT SYSTEM



The membrane attack complex

Jules Bordet

(1870-1961)

1892 – Doctor of Medicine (Brussels)

1894 – He went to Paris to work at the Pasteur Institute until 1901

1898 – He discovered haemolytic sera, and showed that the mechanism of their action on foreign blood is similar to that by which an antimicrobial serum acts on microbes

1899 – He married Marthe Levoz, with whom he had 3 children.

1901 – He returned to Brussels to found the Pasteur Institute

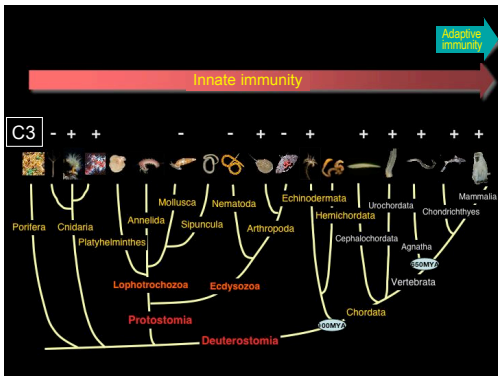
1906 – He isolated the organism that is the bacterial cause of whooping cough : *Bordetella pertussis*.
He also discovered the microbe that causes avian diphtheria and bovine pleuropneumonia

1919 – He invented the Bordet-Wasserman reaction that is used to detect syphilis

1919 – He got the Nobel price 

1920-1940 – He studied bacteriophages

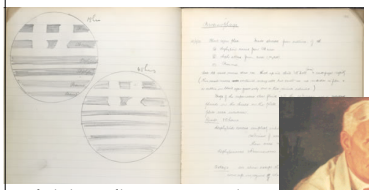
1940 – Bordet retired : his son succeeded him as director at the Pasteur Institute of Brabant



Discovery of lysozyme by Alexander Fleming

In the early 1920s, the British scientist Alexander Fleming reported that a product in human tears, and nasal mucus could lyse bacterial cells.

Fleming's finding, which he called lysozyme, was the first example of an antibacterial agent found in humans.



Notes for the discovery of lysozyme, antiseptic in nasal mucus capable of dissolving bacteria, 21 November 1921 British Library ©

SPITZNAGEL JK, CHI HY.
Cationic proteins and antibacterial properties of infected tissues and leukocytes.
Am J Pathol. 1963 Oct;43:697-711

JOURNAL OF BACTERIOLOGY, Feb., 1966
Copyright © 1966 American Society for Microbiology
Vol. 91, No. 2
Printed in U.S.A.

Cationic Proteins of Polymorphonuclear Leukocyte Lysosomes

I. Resolution of Antibacterial and Enzymatic Activities

H. I. ZEYA AND J. K. SPITZNAGEL[1]
Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina

Defensins

Natural Peptide Antibiotics of Human Neutrophils

Tomas Ganz, Michael E. Selsted, Dorothy Szklarek, Sylvia S. L. Harwig, Kathleen Daher, Dorothy F. Bainton, and Robert I. Lehrer
Departments of Medicine and Pathology, University of California at Los Angeles School of Medicine, University of California at Los Angeles Center for the Health Sciences, Los Angeles, California 90024; and Department of Pathology, University of California-San Francisco School of Medicine, San Francisco, California 94143

J. Clin. Invest.
Volume 76, October 1985, 1427–1435

A Critical Role of Natural Immunoglobulin M in Immediate Defense Against Systemic Bacterial Infection

By Marianne Boes,* Andrey P. Prodeus,‡ Tara Schmidt,* Michael C. Carroll,‡ and Jianzhu Chen*

J. Exp. Med.
Volume 188, Number 12, December 21, 1998 2381–2386

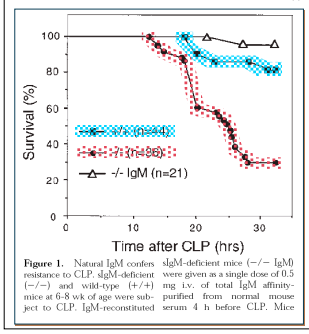
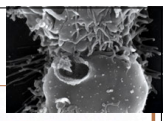


Figure 1. Natural IgM confers resistance to CLP. sIgM-deficient (−/−) and wild-type (+/+) mice at 6–8 wk of age were subject to CLP. IgM-reconstituted sIgM-deficient mice (−/− IgM) were given as a single dose of 0.5 mg i.v. of total IgM affinity-purified from normal mouse serum 4 h before CLP. Mice

Table 1. *Analyses of Peritoneal Lavage 3 h after CLP*

TNF-α (pg/ml)	1,000	912
IL-6 (pg/ml)	1,000	1,001
Neutrophils (%)	0.1	66.8
E. coli (CFU)	550 × 10⁶	550 × 10⁶
LPS (EU/ml)	80	62.9



Transplant Proc. 1971 Mar; 3(1): 915-7.

Thymus-derived Cells as Killer Cells in Cell-mediated Immunity

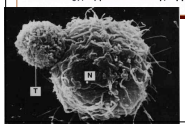
By J. F. A. P. MILLER, K. T. BRUNNER, J. SPRENT, P. J. RUSSELL AND G. F. MITCHELL

112 R. Kiessling, E. Klein and H. Wigzell Eur. J. Immunol. 1975. 5: 112–117

R. Kiessling+, Eva Klein+ and H. Wigzell°
Department of Tumor Biology, Karolinska Institute, Stockholm+ and Department of Immunology, Uppsala University, Uppsala°

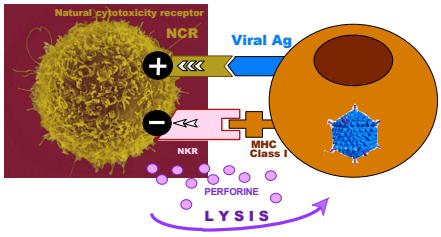
"Natural" killer cells in the mouse

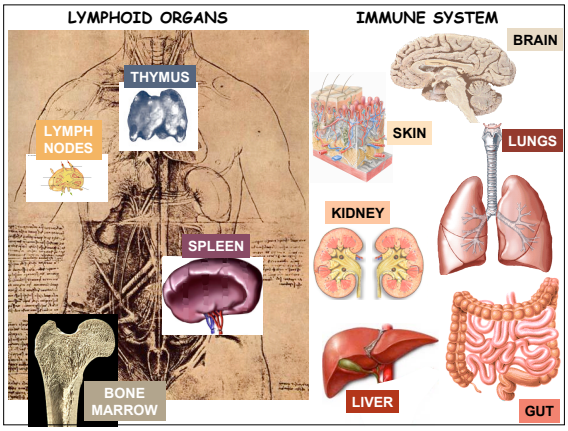
I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype*

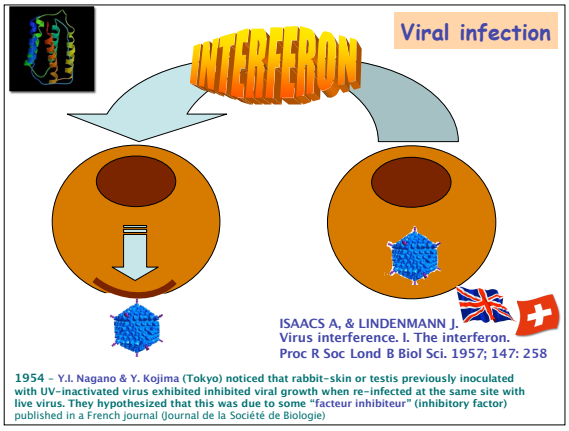


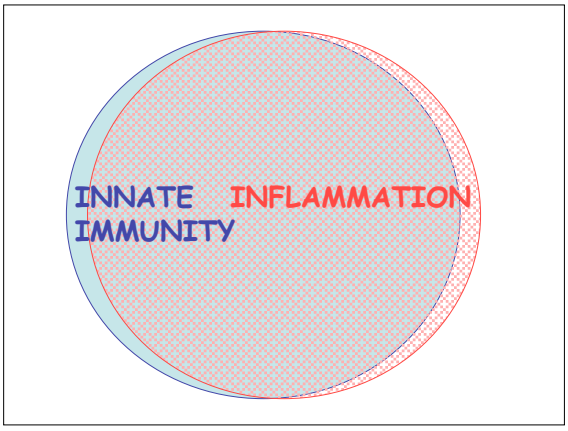
Natural killer cells

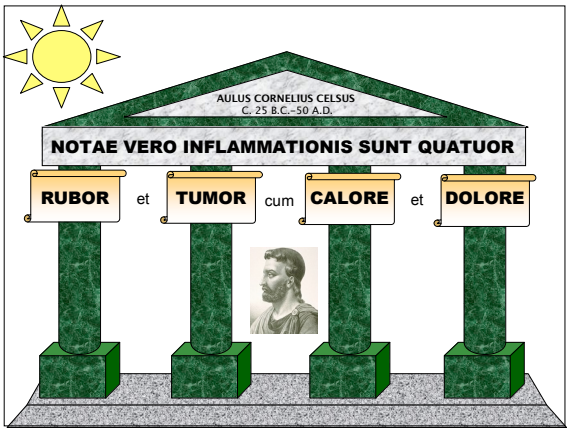
Viral infection

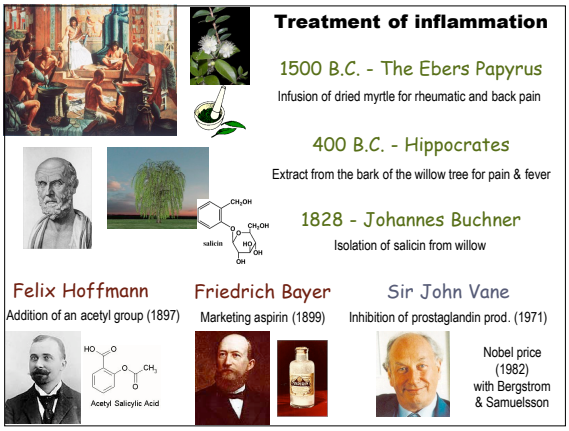


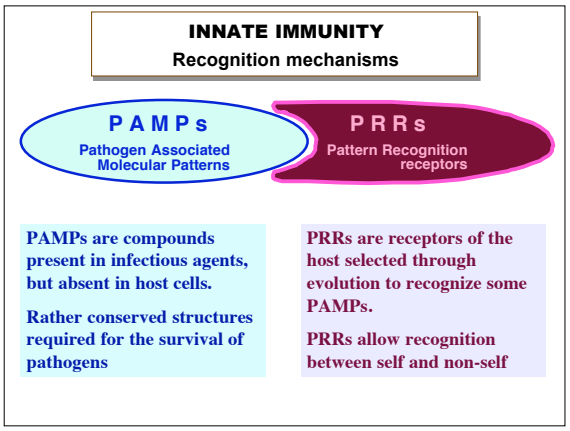


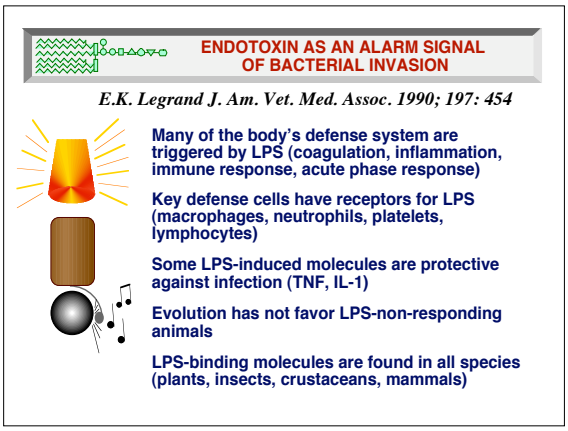

Viral infection
INTERFERON
ISAACS A, & LINDENMANN J.
Virus interference. I. The interferon.
Proc R Soc Lond B Biol Sci. 1957; 147: 258
1954 – Y.I. Nagano & Y. Kojima (Tokyo) noticed that rabbit-skin or testis previously inoculated with UV-inactivated virus exhibited inhibited viral growth when re-infected at the same site with live virus. They hypothesized that this was due to some "facteur inhibiteur" (inhibitory factor) published in a French journal (Journal de la Société de Biologie)


INNATE IMMUNITY
INFLAMMATION


AULUS CORNELIUS CELSUS
C. 25 B.C.–50 A.D.
NOTAE VERO INFLAMMATIONIS SUNT QUATUOR
RUBOR
et
TUMOR
cum
CALORE
et
DOLORE


Treatment of inflammation
1500 B.C. - The Ebers Papyrus
Infusion of dried myrtle for rheumatic and back pain
400 B.C. - Hippocrates
Extract from the bark of the willow tree for pain & fever
1828 - Johannes Buchner
Isolation of salicin from willow
Felix Hoffmann
Addition of an acetyl group (1897)
Acetyl Salicylic Acid
Friedrich Bayer
Marketing aspirin (1899)
Sir John Vane
Inhibition of prostaglandin prod. (1971)
Nobel price (1982) with Bergstrom & Samuelsson


INNATE IMMUNITY
Recognition mechanisms
PAMPs
Pathogen Associated Molecular Patterns
PRRs
Pattern Recognition receptors
PAMPs are compounds present in infectious agents, but absent in host cells.
Rather conserved structures required for the survival of pathogens
PRRs are receptors of the host selected through evolution to recognize some PAMPs.
PRRs allow recognition between self and non-self

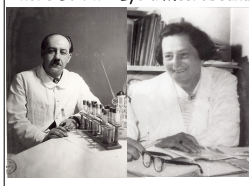

ENDOTOXIN AS AN ALARM SIGNAL OF BACTERIAL INVASION
E.K. Legrand J. Am. Vet. Med. Assoc. 1990; 197: 454
Many of the body's defense system are triggered by LPS (coagulation, inflammation, immune response, acute phase response)
Key defense cells have receptors for LPS (macrophages, neutrophils, platelets, lymphocytes)
Some LPS-induced molecules are protective against infection (TNF, IL-1)
Evolution has not favor LPS-non-responding animals
LPS-binding molecules are found in all species (plants, insects, crustaceans, mammals)

1892 The concept of endotoxin

Richard Pfeiffer

1935 The first biochemical characterization

André Boivin Lydia Mesrobeanu



ARCHIVES ROUMAINES
DE PATHOLOGIE EXPÉRIMENTALE
ET DE MICROBIOLOGIE

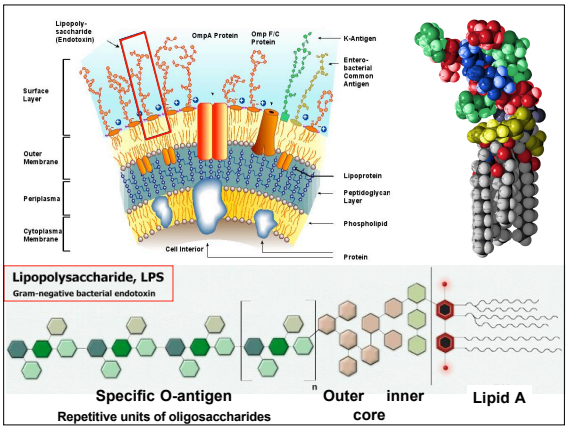
MÉMOIRES ORIGINAUX

LES ANTIGÈNES GLUCIDO-LIPIDIQUES DES BACTÉRIES
(Étude chimique et biologique)

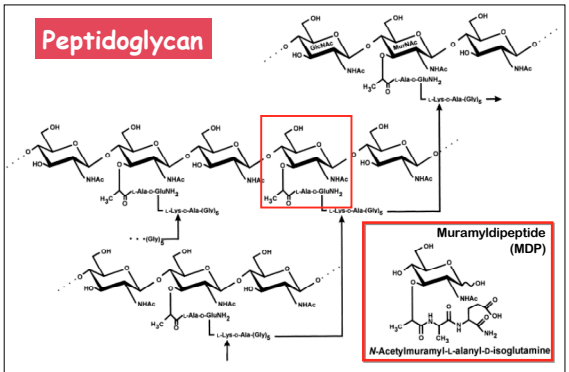
par

Lydia MESROBIANU

1943 Murray Shear : "lipopolysaccharide"



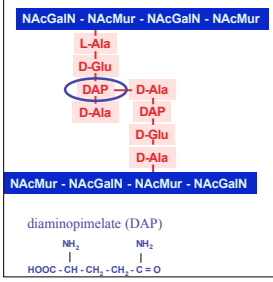
Peptidoglycan



Audibert F, **Chedid Louis**, Lefrancier P, Choay J, **Lederer** Edgar
Relationship between chemical structure and adjuvant activity of some synthetic analogues of N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP).
Ann Immunol (Paris). 1977; 128 C: 653-61.

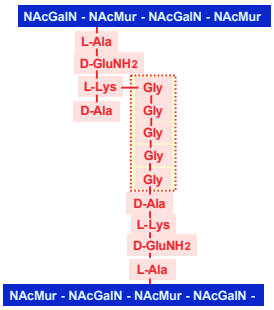
Peptidoglycan

Gram negative

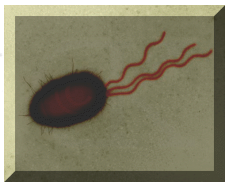


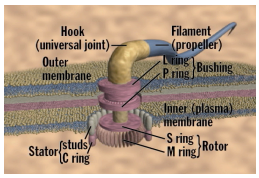
diaminopimelate (DAP)

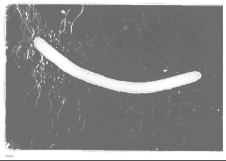
Gram positive



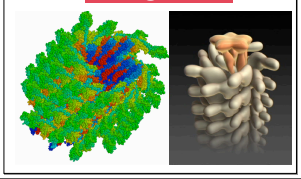
Flagella







Flagellin

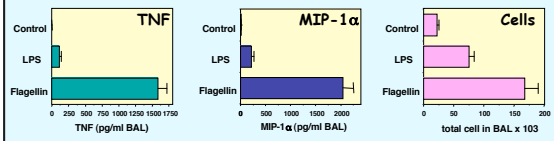


FLAGELLIN IS A POTENT INDUCER OF ACUTE PULMONARY INFLAMMATION IN SEPSIS

Liaudet et al. Shock 2003, 19, 131

MOUSE MODEL

Measurements performed in BAL, 4h after 100 µg i.v. injection of *Salmonella* LPS or Flagellin



HUMAN SEPSIS

Serum

Mean Flagellin = 7.1 ± 0.1 ng/ml

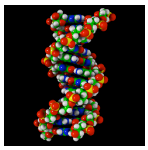
(range = 2 - 20 ng/ml)

Correlations

Duration of septic shock
r = 0.71 , p = 0.002

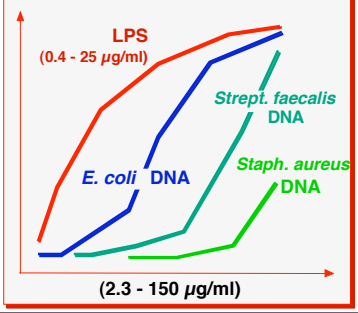
Lung injury score
r = 0.59 , p = 0.017

BACTERIAL DNA CAUSES SEPTIC SHOCK



Sparwasser et al. Nature 1997, 386, 336

TNF production by ANA-1 MØ cell line



Gram positive and Gram negative DNA causes lethal toxic shock in galactosamine sensitized mice

in contrats to bacterial DNA, in eukaryotic DNA, the dinucleotide motif 5'-CpG-3' is mostly methylated

~~NON SPECIFIC IMMUNITY~~

INNATE IMMUNITY

RECEPTORS	**Fixed in the genome**
RECOGNITION	**Conserved molecular patterns**
SELF & NON-SELF DISCRIMINATION	**Perfect : selected over evolutionary time**
ACTION TIME	**Immediate activation of effectors**
RESPONSE	**Costimulatory molecules : cytokines, chemokines**

From C.A. Janeway J. Immunol. 1998, 161, 544

INNATE IMMUNITY OF DROSOPHILA

Physical and chemical barriers

Melanization & coagulation

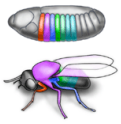
Phagocytosis et encapsulation

Production of antimicrobial peptides

fatty body cells

- **Drosomycin**
- **Drosocin**
- **Defensin**
- **Cecropin**
- **Attacin**
- **Diptericin**
- **Metchnikowin**

Christiane Nüsslein-Volhard



Anderson KV, Jurgens G, Nusslein-Volhard C.

Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product.

Cell. 1985 Oct;42(3):779-89.

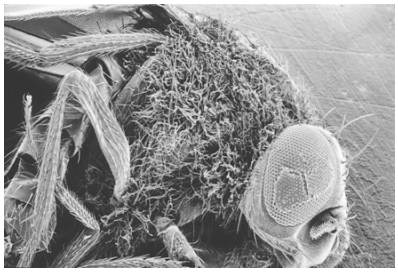
The Nobel Prize in Physiology or Medicine 1995

"for their discoveries concerning the genetic control of early embryonic development"

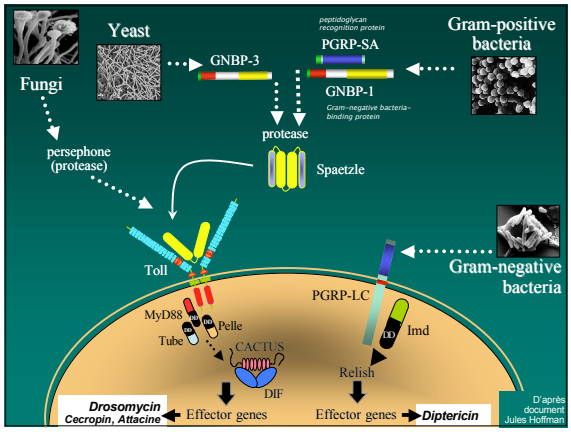
Toll receptor in *Drosophila*

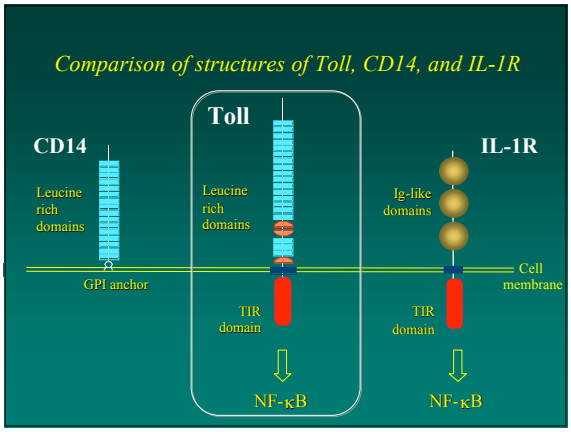
Involved in embryological development

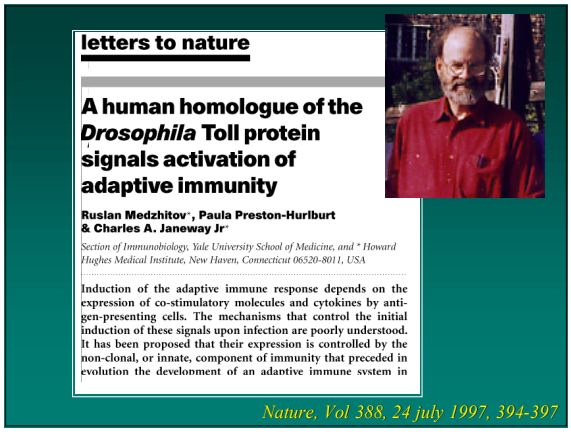
Involved in the immune response against fungal infection

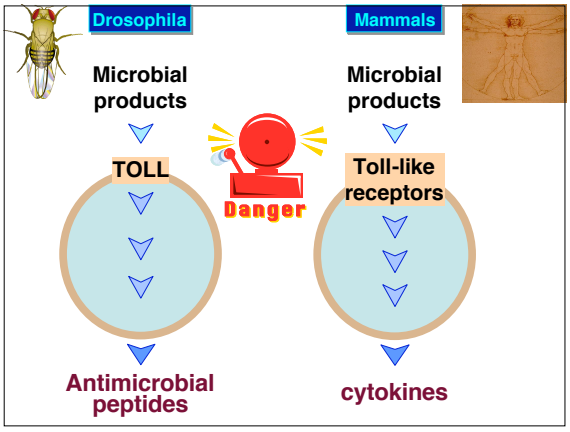


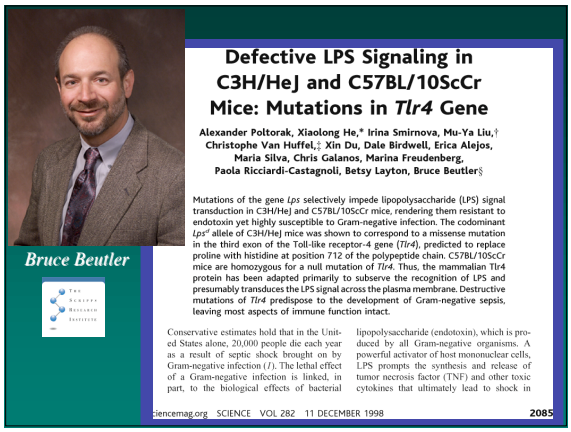
Lemaitre et al. Cell 1996, 86:973

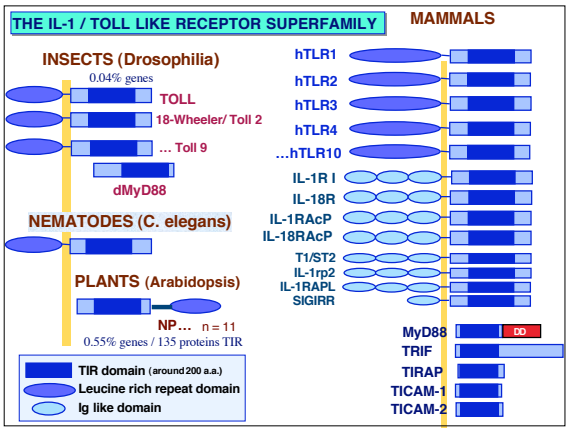


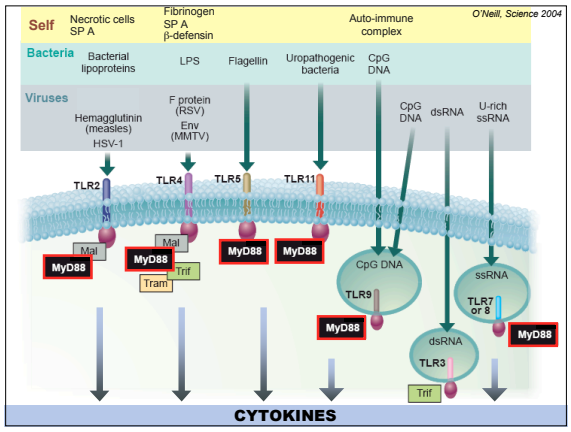

Comparison of structures of Toll, CD14, and IL-1R
CD14
Toll
IL-1R
Leucine rich domains
Leucine rich domains
Ig-like domains
GPI anchor
Cell membrane
TIR domain
TIR domain
NF-κB
NF-κB

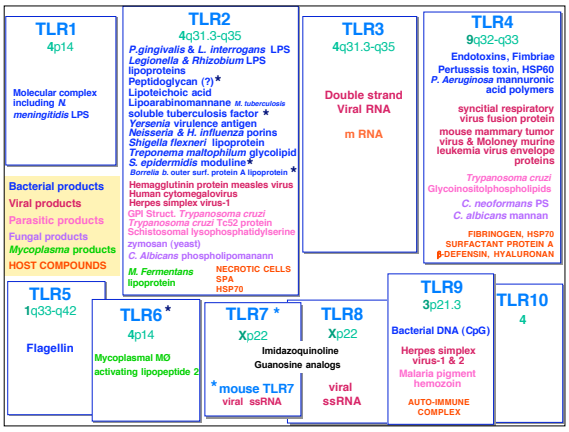

letters to nature
A human homologue of the Drosophila Toll protein signals activation of adaptive immunity
Ruslan Medzhitov*, Paula Preston-Hurlburt & Charles A. Janeway Jr*
Section of Immunobiology, Yale University School of Medicine, and * Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011, USA
Induction of the adaptive immune response depends on the expression of co-stimulatory molecules and cytokines by antigen-presenting cells. The mechanisms that control the initial induction of these signals upon infection are poorly understood. It has been proposed that their expression is controlled by the non-clonal, or innate, component of immunity that preceded in evolution the development of an adaptive immune system in
Nature, Vol 388, 24 july 1997, 394-397

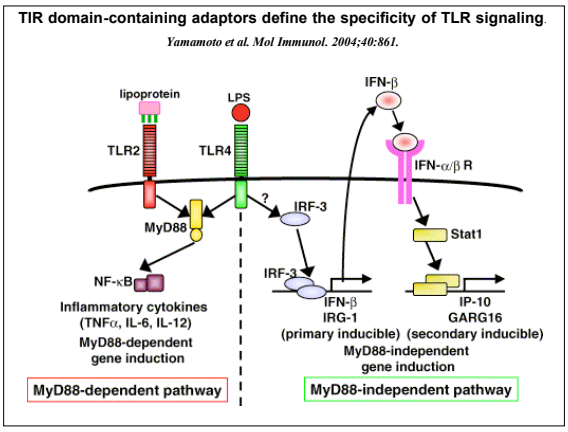

Drosophila
Mammals
Microbial products
Microbial products
TOLL
Danger
Toll-like receptors
Antimicrobial peptides
cytokines

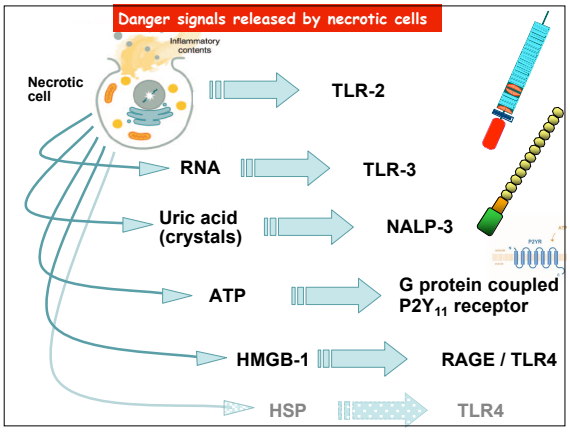

Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in Tlr4 Gene
Alexander Poltorak, Xiaolong He,* Irina Smirnova, Mu-Ya Liu,† Christophe Van Huffel,‡ Xin Du, Dale Birdwell, Erica Alejos, Maria Silva, Chris Galanos, Marina Freudenberg, Paola Ricciardi-Castagnoli, Betsy Layton, Bruce Beutler§
Bruce Beutler
Mutations of the gene Lps selectively impede lipopolysaccharide (LPS) signal transduction in C3H/HeJ and C57BL/10ScCr mice, rendering them resistant to endotoxin yet highly susceptible to Gram-negative infection. The codominant Lps^d allele of C3H/HeJ mice was shown to correspond to a missense mutation in the third exon of the Toll-like receptor-4 gene (Tlr4), predicted to replace proline with histidine at position 712 of the polypeptide chain. C57BL/10ScCr mice are homozygous for a null mutation of Tlr4. Thus, the mammalian Tlr4 protein has been adapted primarily to subserve the recognition of LPS and presumably transduces the LPS signal across the plasma membrane. Destructive mutations of Tlr4 predispose to the development of Gram-negative sepsis, leaving most aspects of immune function intact.
Conservative estimates hold that in the United States alone, 20,000 people die each year as a result of septic shock brought on by Gram-negative infection (1). The lethal effect of a Gram-negative infection is linked, in part, to the biological effects of bacterial lipopolysaccharide (endotoxin), which is produced by all Gram-negative organisms. A powerful activator of host mononuclear cells, LPS prompts the synthesis and release of tumor necrosis factor (TNF) and other toxic cytokines that ultimately lead to shock in
sciencemag.org SCIENCE VOL 282 11 DECEMBER 1998
2085


THE IL-1 / TOLL LIKE RECEPTOR SUPERFAMILY
MAMMALS
INSECTS (Drosophila)
0.04% genes
TOLL
18-Wheeler/ Toll 2
... Toll 9
dMyD88
NEMATODES (C. elegans)
PLANTS (Arabidopsis)
NP... n = 11
0.55% genes / 135 proteins TIR
hTLR1
hTLR2
hTLR3
hTLR4
...hTLR10
IL-1R I
IL-18R
IL-1RAcP
IL-18RAcP
T1/ST2
IL-1rp2
IL-1RAPL
SIGIRR
MyD88
TRIF
TIRAP
TICAM-1
TICAM-2
DD
TIR domain (around 200 a.a.)
Leucine rich repeat domain
Ig like domain


O'Neill, Science 2004
Self
Necrotic cells
SP A
Fibrinogen
SP A
β-defensin
Auto-immune complex
Bacteria
Bacterial lipoproteins
LPS
Flagellin
Uropathogenic bacteria
CpG DNA
Viruses
Hemagglutinin (measles)
HSV-1
F protein (RSV)
Env (MMTV)
CpG DNA
dsRNA
U-rich ssRNA
TLR2
TLR4
TLR5
TLR11
Mal
MyD88
Trif
Tram
CpG DNA
TLR9
ssRNA
TLR7 or 8
dsRNA
TLR3
CYTOKINES


TLR1
4p14
Molecular complex including N meningitidis LPS
TLR2
4q31.3-q35
P.gingivalis & L. interrogans LPS
Legionella & Rhizobium LPS
lipoproteins
Peptidoglycan (?)*
Lipoteichoic acid
Lipoarabinomannane M. tuberculosis
soluble tuberculosis factor *
Yersenia virulence antigen
Neisseria & H. influenza porins
Shigella flexneri lipoprotein
Treponema maltophilum glycolipid
S. epidermidis moduline*
Borrelia b. outer surf. protein A lipoprotein *
Hemagglutinin protein measles virus
Human cytomegalovirus
Herpes simplex virus-1
GPI Struct. Trypanosoma cruzi
Trypanosoma cruzi Tc52 protein
Schistosomal lysophosphatidylserine
zymosan (yeast)
C. Albicans phospholipomanann
M. Fermentans lipoprotein
NECROTIC CELLS
SPA
HSP70
TLR3
4q31.3-q35
Double strand Viral RNA
m RNA
TLR4
9q32-q33
Endotoxins, Fimbriae
Pertussis toxin, HSP60
P. Aeruginosa mannuronic acid polymers
syncitial respiratory virus fusion protein
mouse mammary tumor virus & Moloney murine leukemia virus envelope proteins
Trypanosoma cruzi
Glycoinositolphospholipids
C. neoformans PS
C. albicans mannan
FIBRINOGEN, HSP70
SURFACTANT PROTEIN A
β-DEFENSIN, HYALURONAN
Bacterial products
Viral products
Parasitic products
Fungal products
Mycoplasma products
HOST COMPOUNDS
TLR5
1q33-q42
Flagellin
TLR6 *
4p14
Mycoplasmal MØ activating lipopeptide 2
TLR7 *
Xp22
Imidazoquinoline
Guanosine analogs
* mouse TLR7
viral ssRNA
TLR8
Xp22
viral ssRNA
TLR9
3p21.3
Bacterial DNA (CpG)
Herpes simplex virus-1 & 2
Malaria pigment hemozoin
AUTO-IMMUNE COMPLEX
TLR10
4


TIR domain-containing adaptors define the specificity of TLR signaling.
Yamamoto et al. Mol Immunol. 2004;40:861.
lipoprotein
LPS
IFN-β
TLR2
TLR4
IFN-α/β R
?
IRF-3
MyD88
Stat1
NF-κB
IRF-3
Inflammatory cytokines
(TNFα, IL-6, IL-12)
MyD88-dependent
gene induction
IFN-β
IRG-1
(primary inducible)
IP-10
GARG16
(secondary inducible)
MyD88-independent
gene induction
MyD88-dependent pathway
MyD88-independent pathway


Danger signals released by necrotic cells
Inflammatory contents
Necrotic cell
TLR-2
RNA
TLR-3
Uric acid (crystals)
NALP-3
ATP
G protein coupled
P2Y11 receptor
HMGB-1
RAGE / TLR4
HSP
TLR4