The global struggle against infectious disease

5th ASEAN "Bridges" Event, Sponsored by the
International Peace Foundation and Naresuan University

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Bruce Beutler, M.D.

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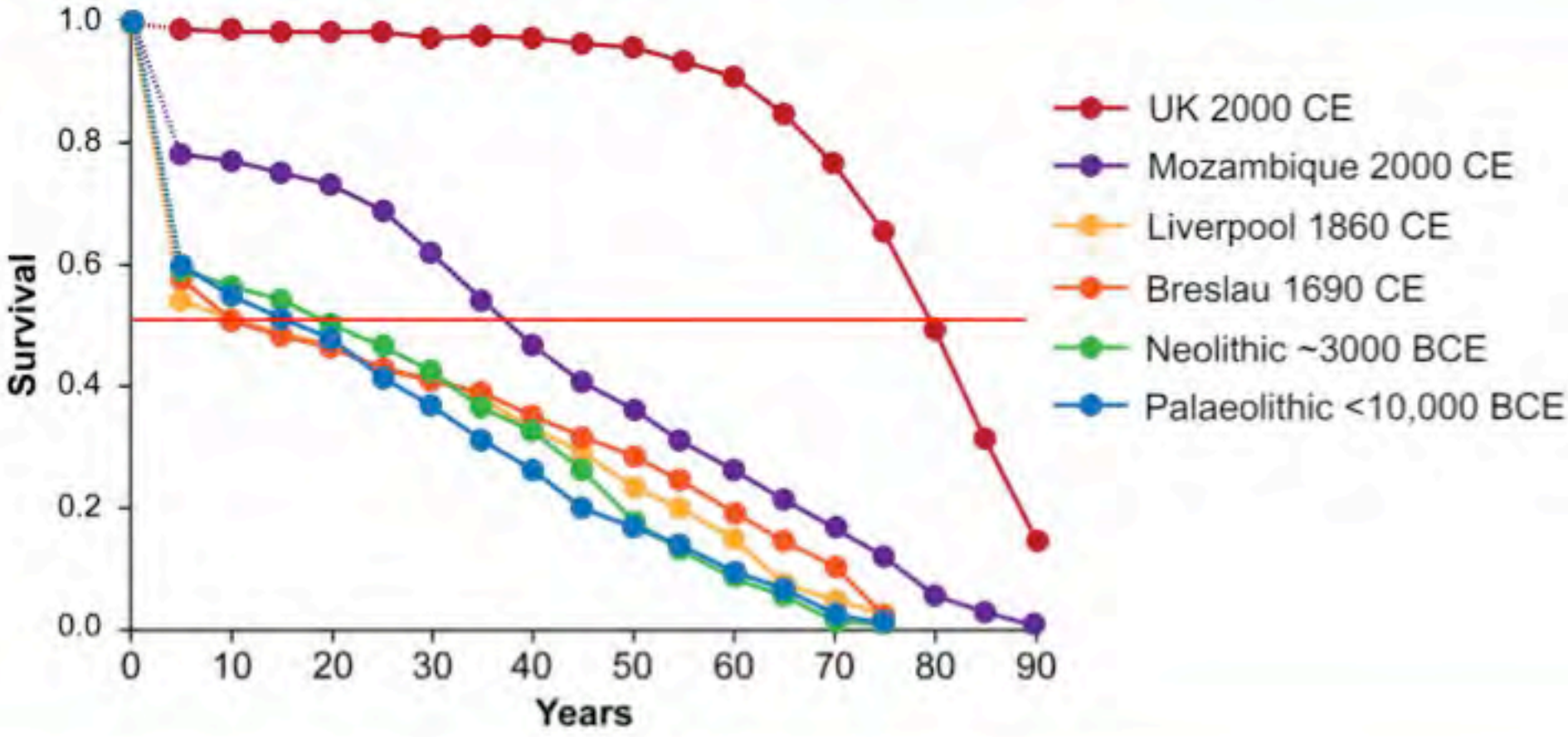
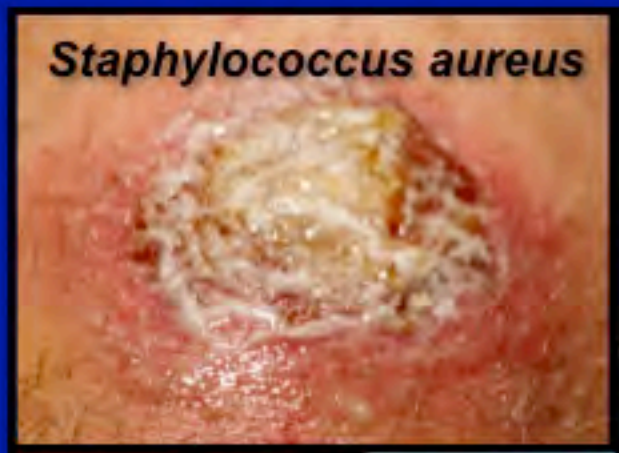
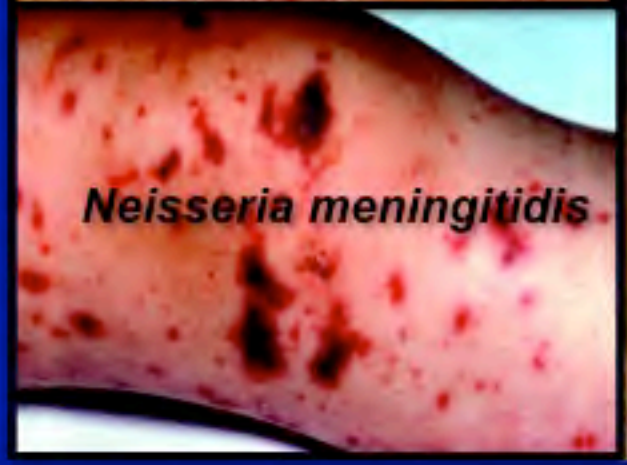
What we die of, and the age to which we live, depends very much on when and where we live.

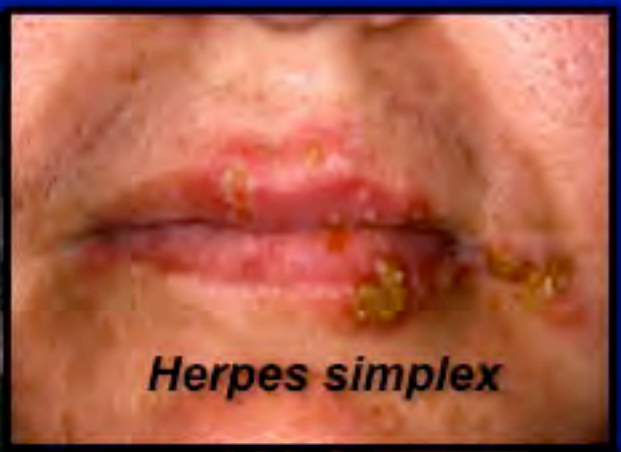


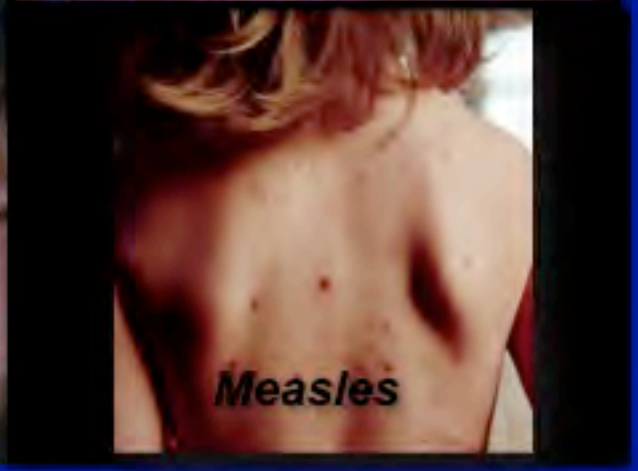
Courtesy J-L Casanova

Infections have diverse effects, but there are common themes in our responses to them. Infections have stolen more years of human life than any other cause.









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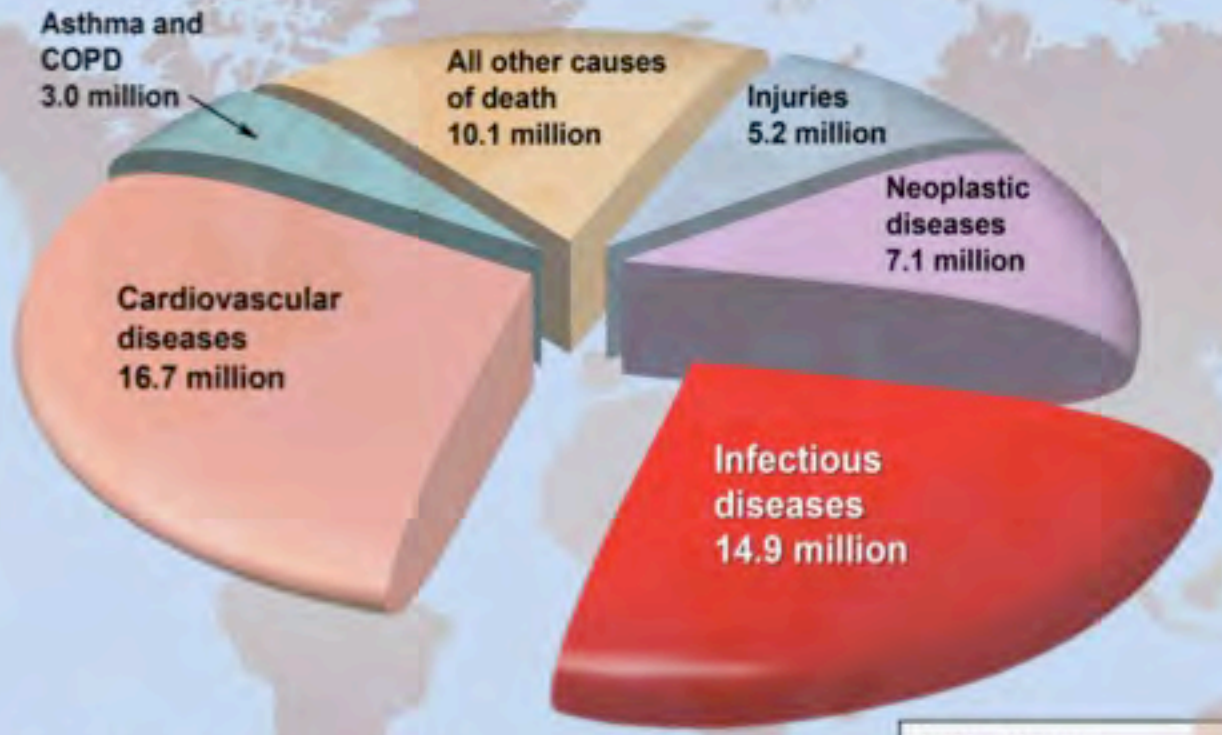


59 million people die each year of all causes, worldwide. Infection accounts for about a quarter of all deaths.

Infectious Diseases	Annual deaths (millions)
Respiratory infections	3.96
HIV/AIDS	2.77
Diarrhoeal diseases	1.90
Tuberculosis	1.56
Vaccine-preventable childhood diseases	1.12
Malaria	1.27
STD's (other than HIV)	0.18
Meningitis	0.17
Hepatitis B and C	0.16
Tropical parasitic deseases	0.13
Dengue	0.02
Other infectious disease	1.76

Based on: The challenge of emerging and re-emerging infectious diseases. D.M. Morens, G.K. Folkers, and A. S. Fauci, Nature **463**, 122(7 January 2010)

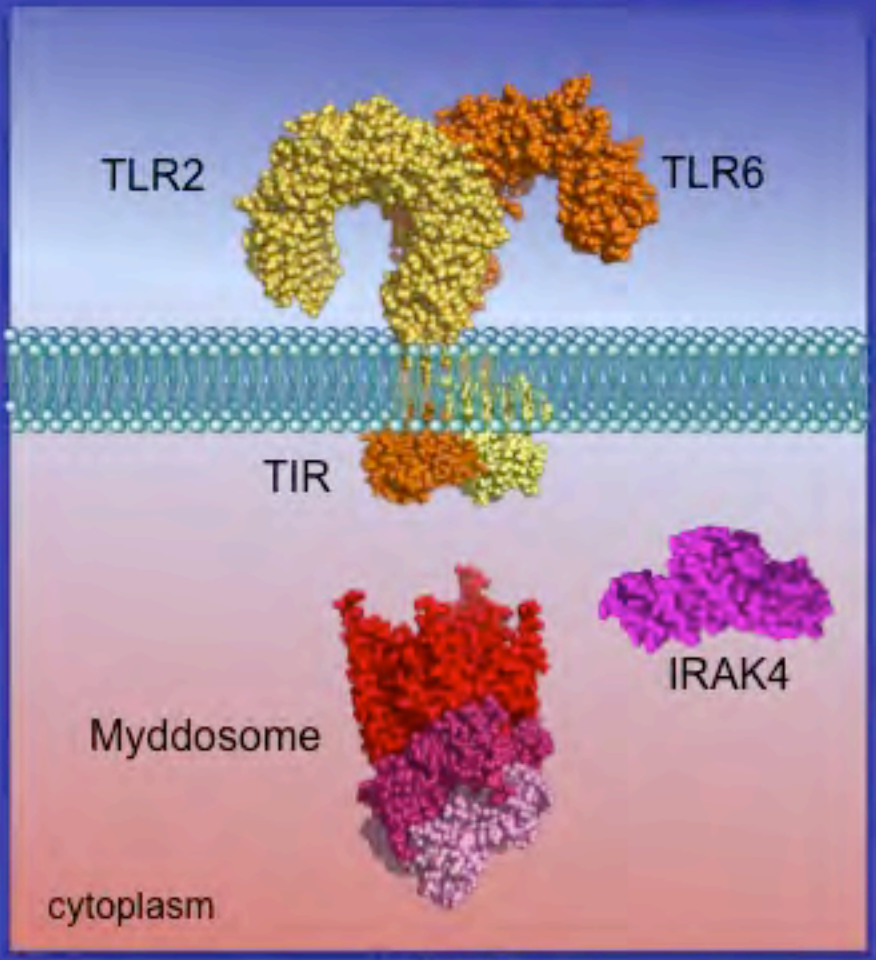
What has the selective pressure of infectious disease done to our species? And to all species?

- We have only been aware of microbes as the cause of infection for about 150 years, but the battle with microbes has gone on for a billion years or more.

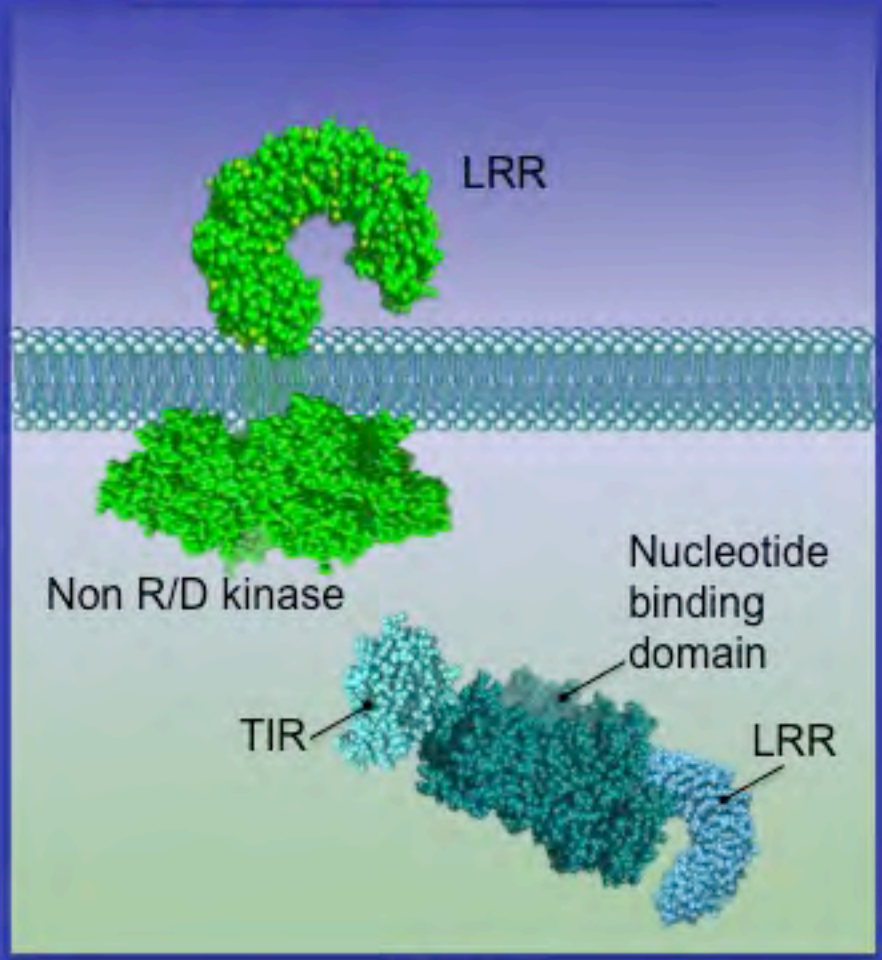
How did immunity evolve?

- Anti-microbial peptides and antibiotics may have been the earliest agents used by microbes to combat one another, and by early eukaryotic organisms to combat microbes. But it's expensive to maintain such a system constitutively. Therefore sensing, signaling components followed soon after.
- As multicellular organisms developed, specialized cells capable of phagocytosis became competent to dispose of microbial invaders. This was the origin of innate immunity, which persists in many life forms today, including mammals, and is essential for survival.
- Components of a sensing and signaling apparatus seem to have pre-dated the split between animals and plants: LRR domain receptors, TIR domains, and NOD-like receptors exist in both.

Innate immune receptors in animals and plants



Animal



Plant

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Timeline: Precambrian to Present

Animalia

Plantae

760 740 720 700 680 660

Cryogenian

Proterozoic

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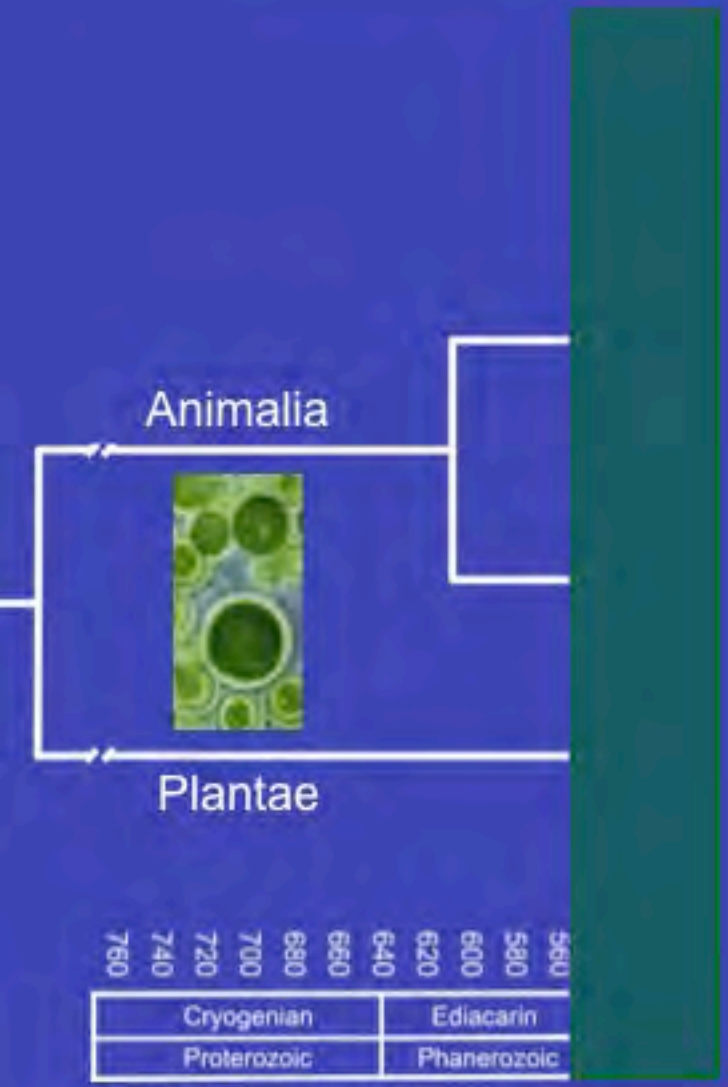
Timeline: Precambrian to Present



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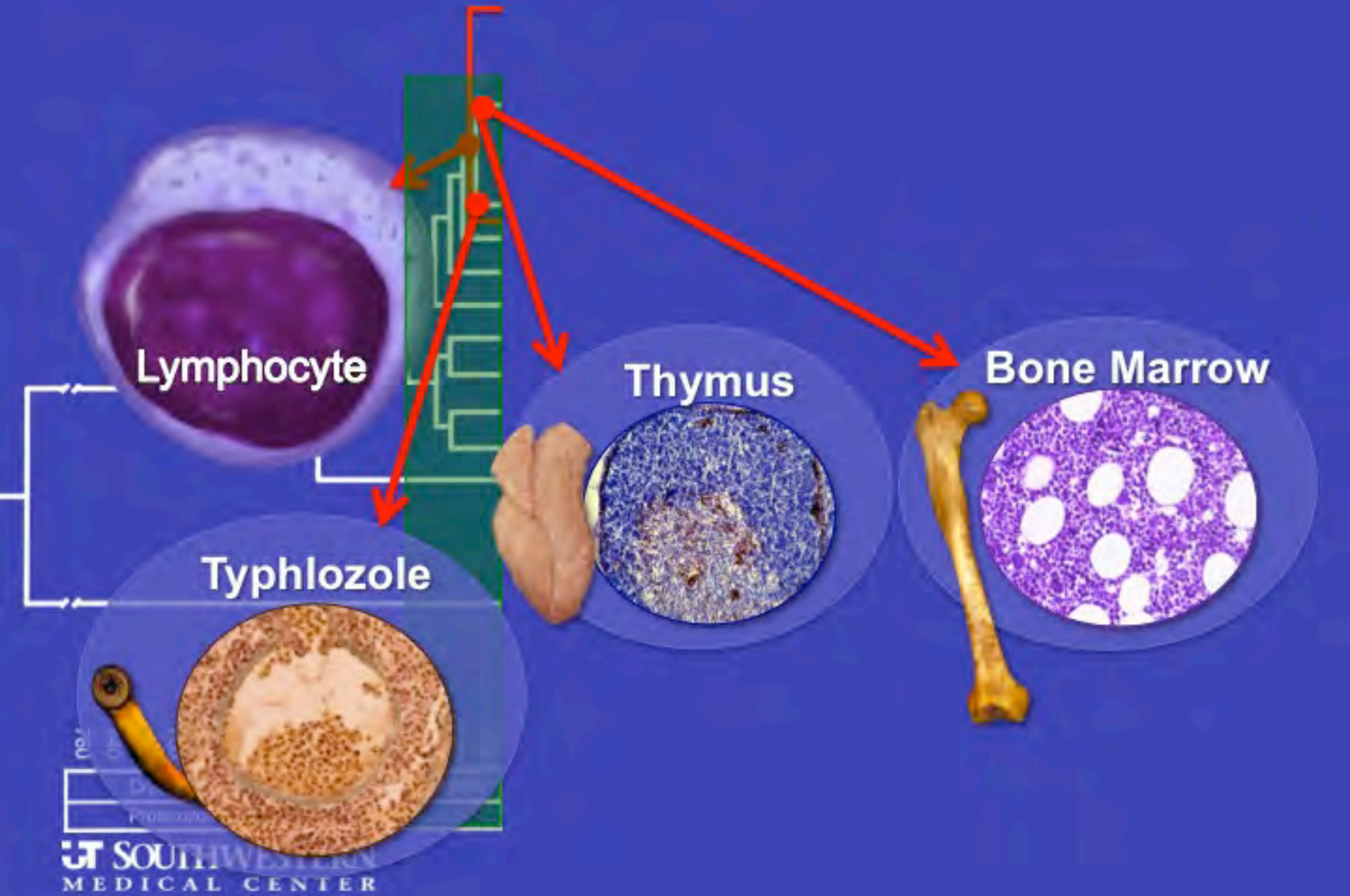
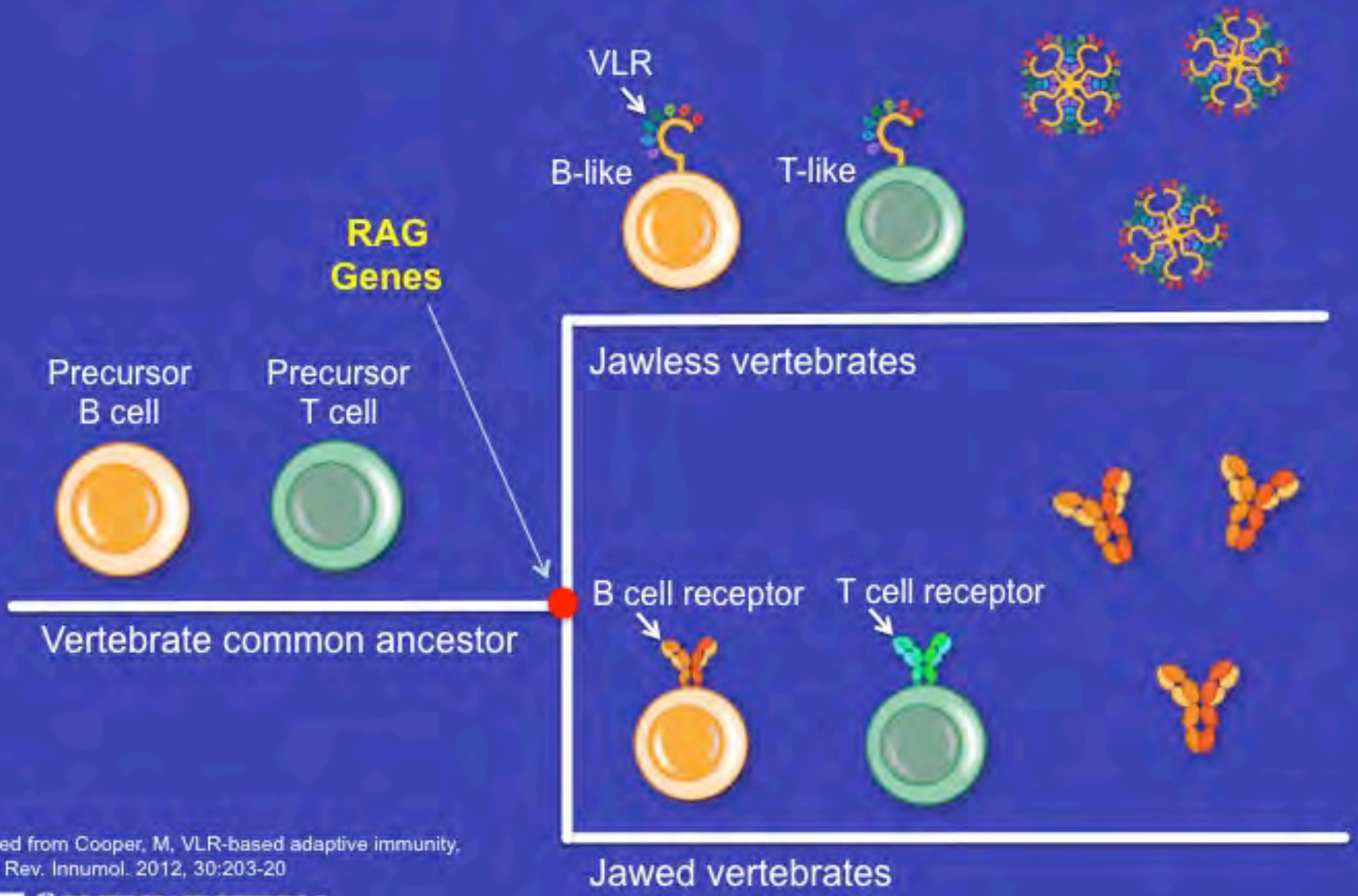
Timeline: Precambrian to Present



Evolution of Adaptive Immunity



Adapted from Cooper, M, VLR-based adaptive immunity, Annu. Rev. Immunol. 2012, 30:203-20

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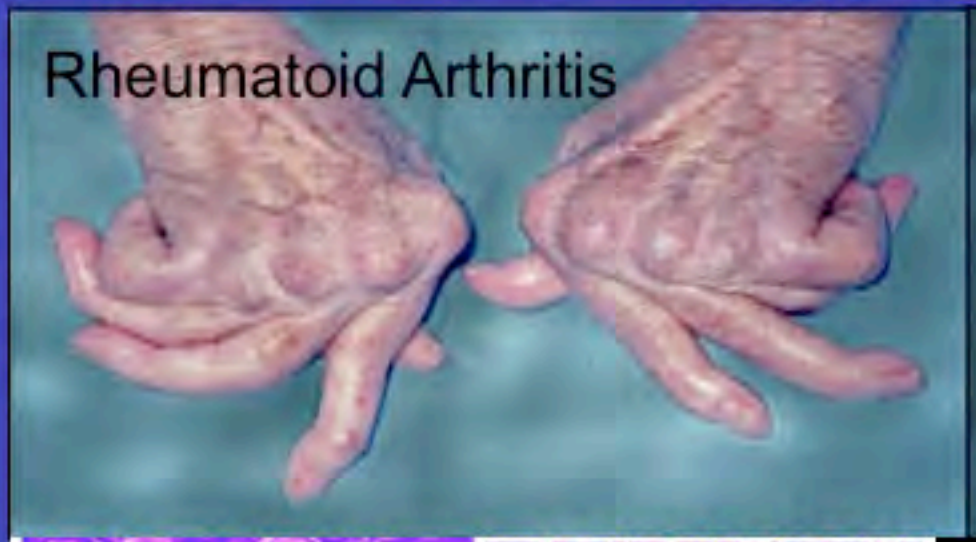
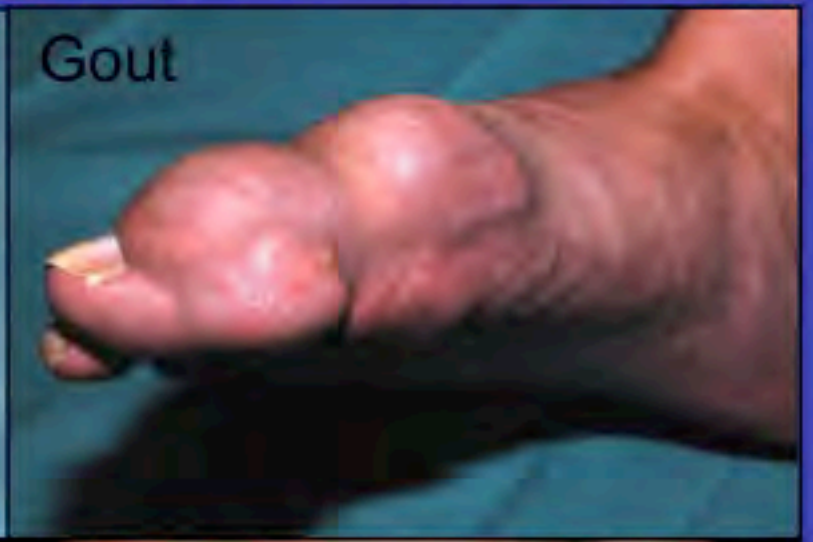
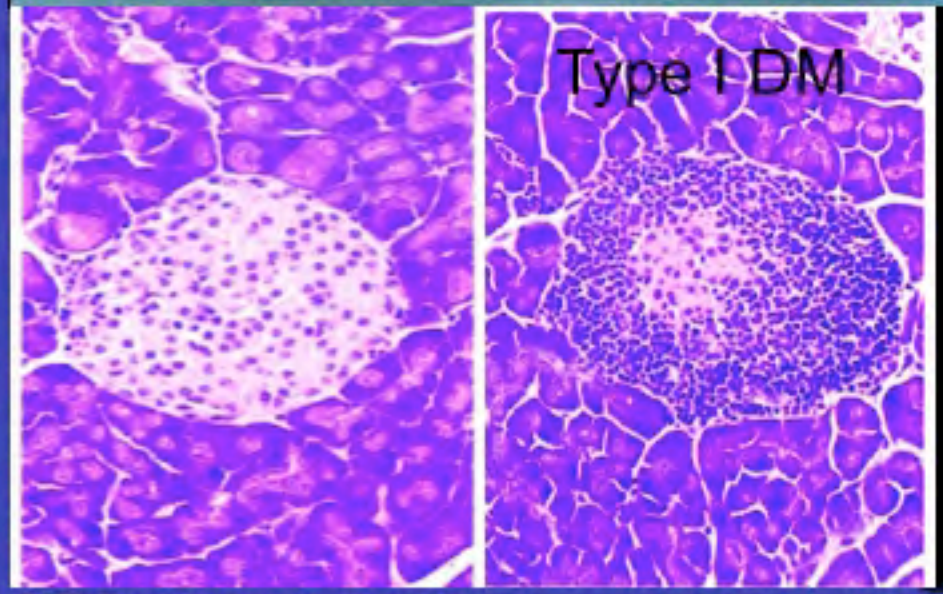
The legacy of our struggle against microbes is immunity.

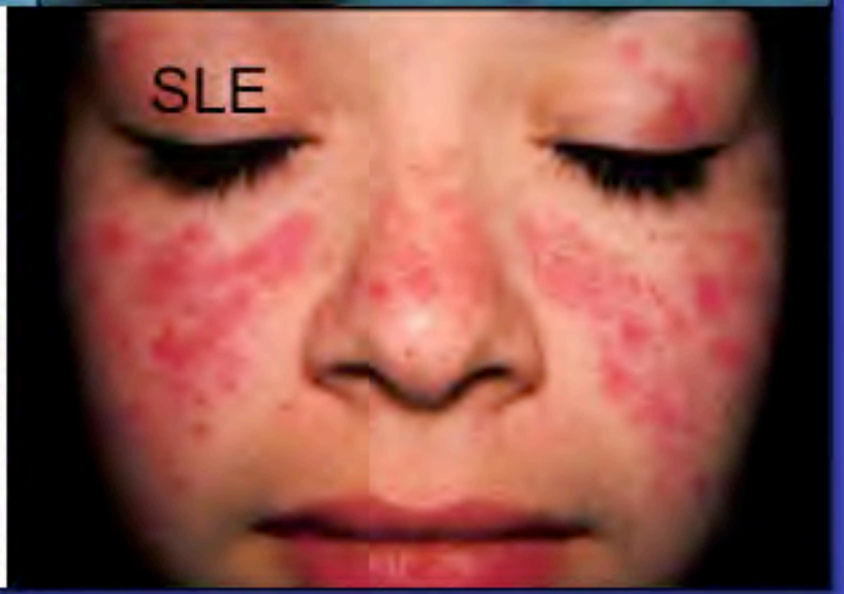
One legacy of immunity is autoimmunity.

Innate and Adaptive Autoimmunity









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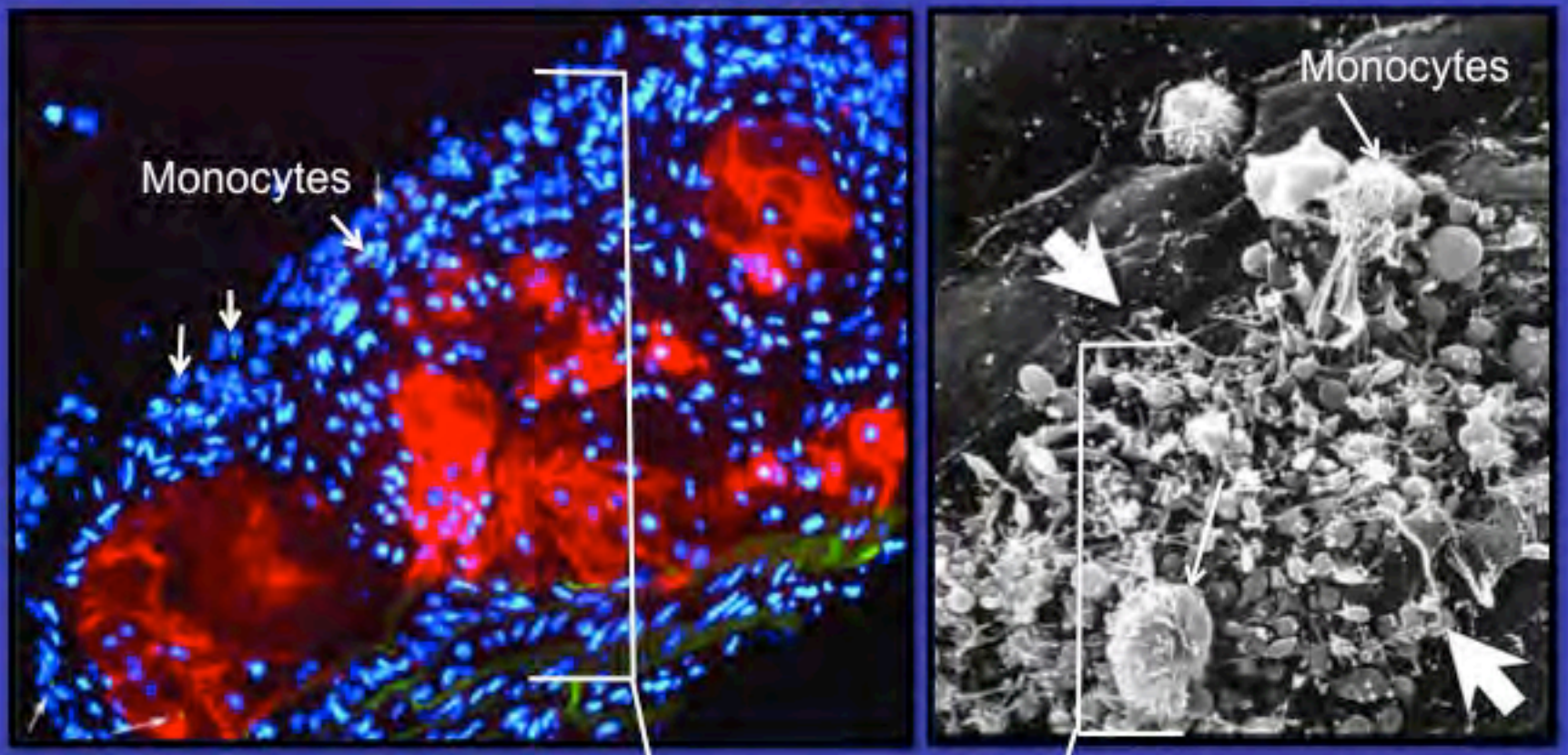
More subtle forms of inflammation: atherosclerotic disease



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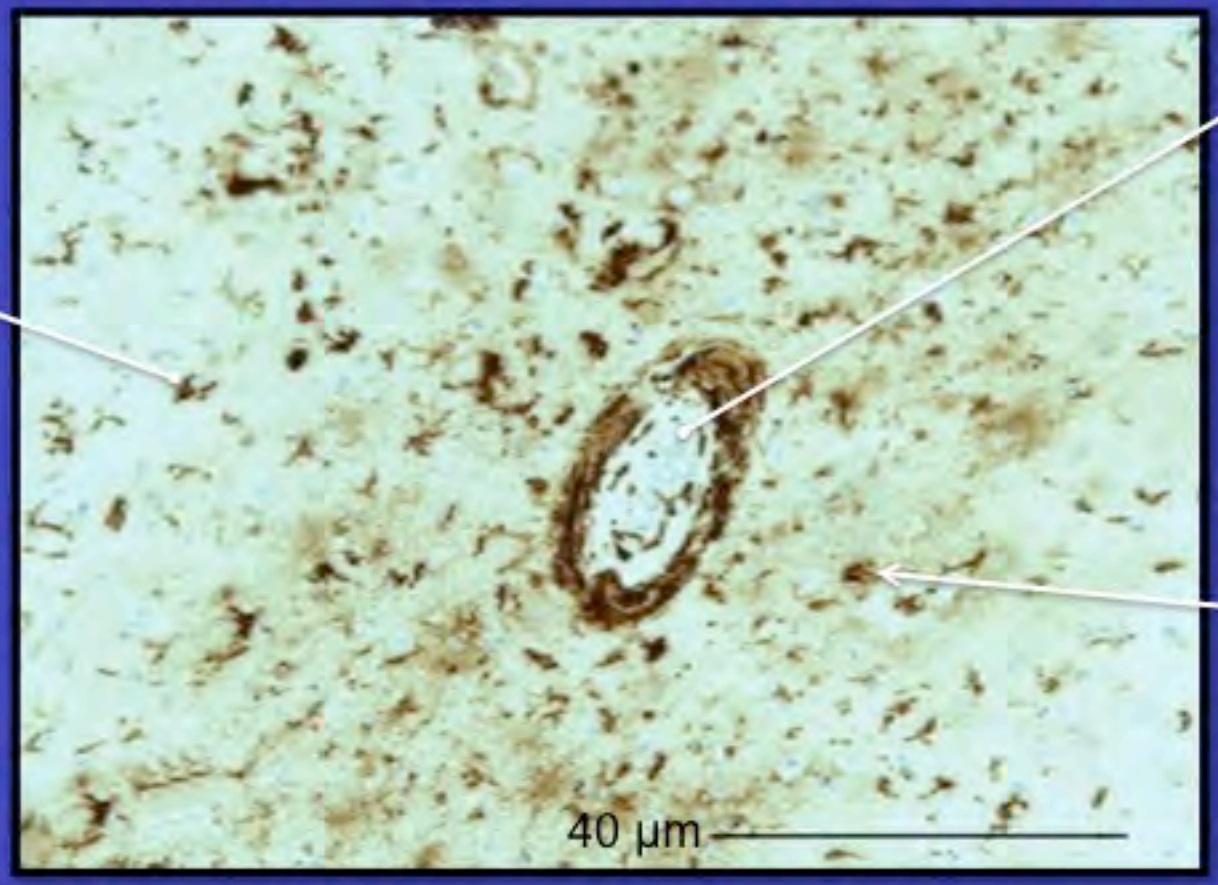
More subtle forms of inflammation: neurodegenerative disease

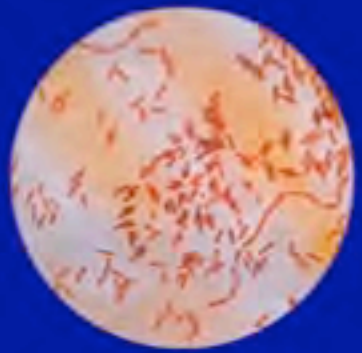


Cerebral cortex stained for HLA-II

Major causes of death in Victorian England

- Smallpox

- Typhoid



- Tuberculosis

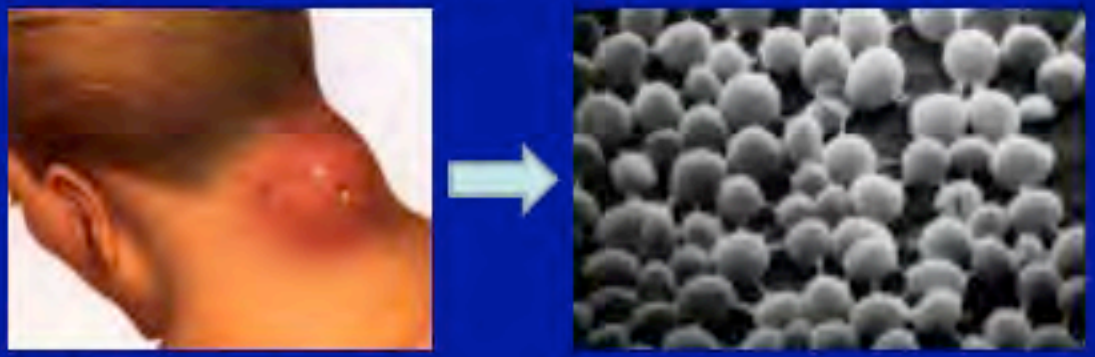
- Cholera


Sarah Nelmes
Edward Jenner
James Phipps
14 May 1796

The Cow-Pock — or — the Wonderful Effects of the New Inoculation! — Vide the Publications of the Anti Vaccine Society.

All approaches to infection were hampered by ignorance as to what infections actually were.

- The link between infection and microbes had yet to be made.



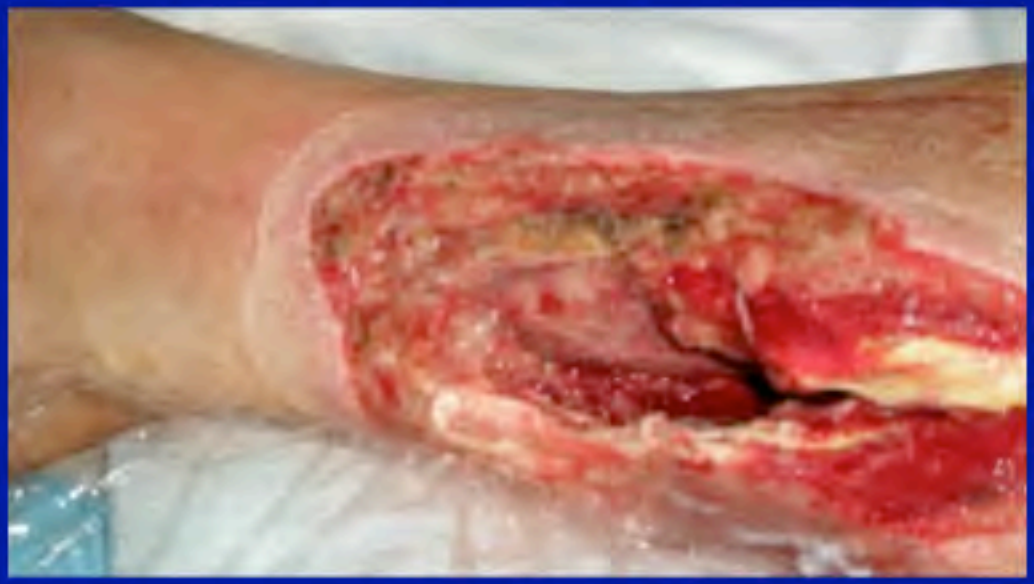
- Until it was made, there were simply vague ideas about how infections spread between individuals, and how they produced effects akin to poisoning.

Miasma

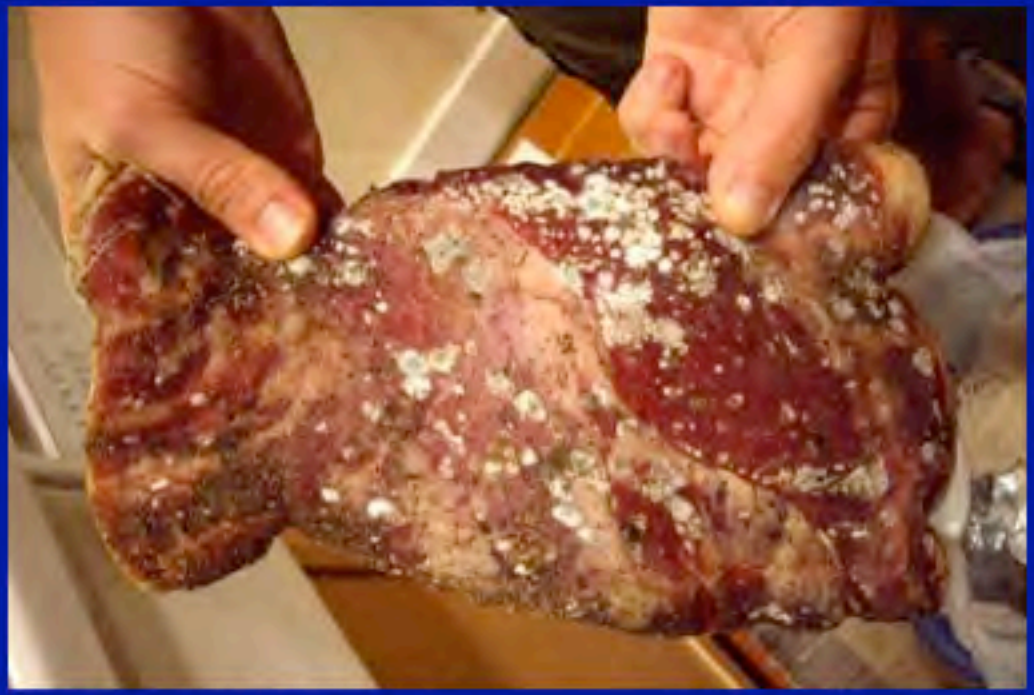
vs.

Contagion

The Selfish Slaves of Doses of Disease and Death.



Generally speaking, infection resembled putrefaction of organic materials (decaying meat or plant matter) with the production of foul smelling gases such as hydrogen sulfide, ammonia, mercaptans



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Putrid materials of plant and animal origin are toxic when injected into animals, eliciting fever

Albrecht von Haller (1708-1777)

François Magendie (1783-1855)

Attempts to purify the “putrid poison”

Ernst von Bergmann (1836-1906)

Theodor Billroth (1829-1894)

Peter Panum (1820-1885)

Peter Panum
(1820-1885)

Peter Panum, 1874 (writing of his own work, performed in 1856):

The putrid poison is not volatile and is not a known simple end product of putrefaction or fermentation. It can be differentiated from living microorganisms, which may be a source but not the cause.

The toxin resists heat and, thus, differs from typical enzymes (at that time called in German *Fermente*).

It is insoluble in pure alcohol but soluble in water.

The protein-like substances frequently present in putrid fluids are not toxic by themselves, but they absorb ("condense") the toxin on their surface when precipated. The toxic principle can be, at least partially, eluted from the precipitates.

Injection of 12 mg of the concentrate suffices to produce high fever and kill a dog.

The Germ Theory of Infectious Diseases

Louis Pasteur
(1822-1895)

Robert Koch
(1843-1910)

France

vs.

Germany

"In his Geneva lecture, Pasteur bitterly complained about my having rejected his microscopic examinations and inoculation techniques. However, after his inoculations with saliva and nose slime and his repeated discovery of the microbe *en huit*, I am not able to change my opinion. Pasteur deserves criticism not only for his defective methods, but also for the way in which he has publicized his investigations."

Robert Koch

Louis Pasteur

"I find here, Monsieur, a new example of the manner of discourse that served you previously in 1881; you attribute to me some errors which I hadn't committed; you refute them and then exult noisily."

One after another, infectious diseases were ascribed to microbes during the 19th and 20th centuries.

- The germ theory of infectious disease became widely accepted.
- The question arose as to how germs are initially recognized by the immune system, and how contact between microbe and host could lead to disease… or to an immune response.
- Questions of microbial pathogenesis arose concurrent with immunology.

Pasteur

Koch

Metchnikoff

Besredka

Ehrlich

Von Behring

Pfeiffer

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Soon after microbes were discovered, it was appreciated that mammals recognize them as foreign and mount an intense inflammatory response

d Pfeiffer (27
15 September
nt of Robert
at heat-killed
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injected into
He coined the
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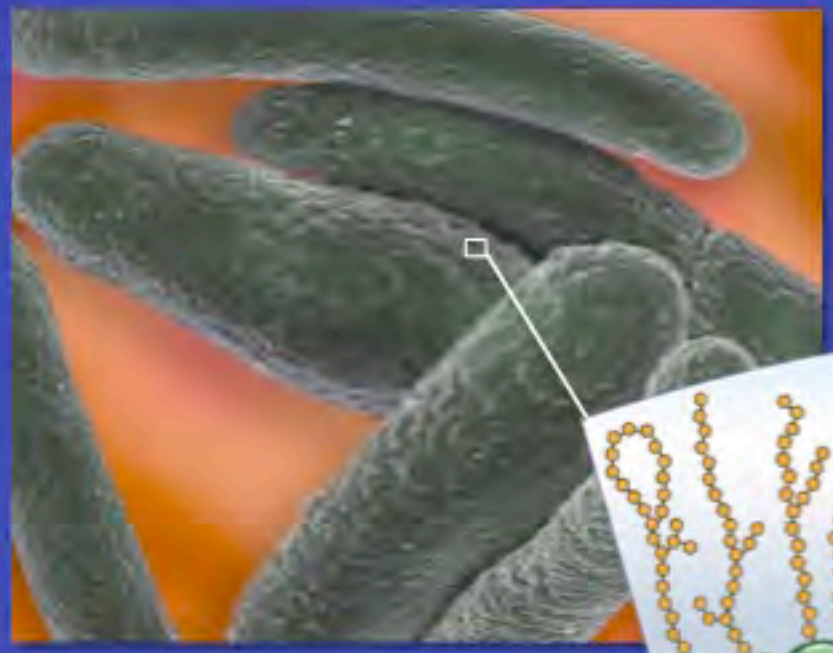
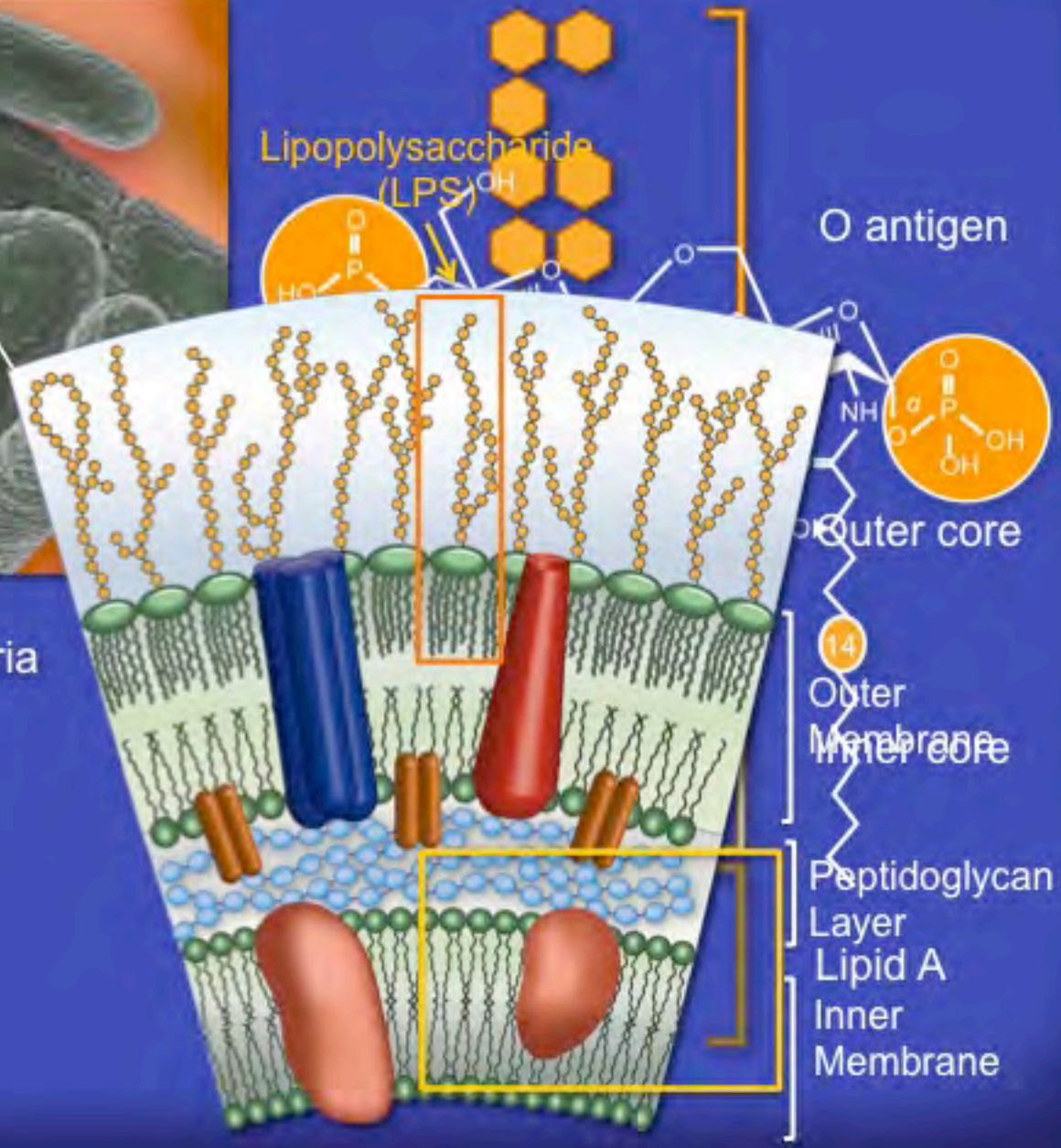
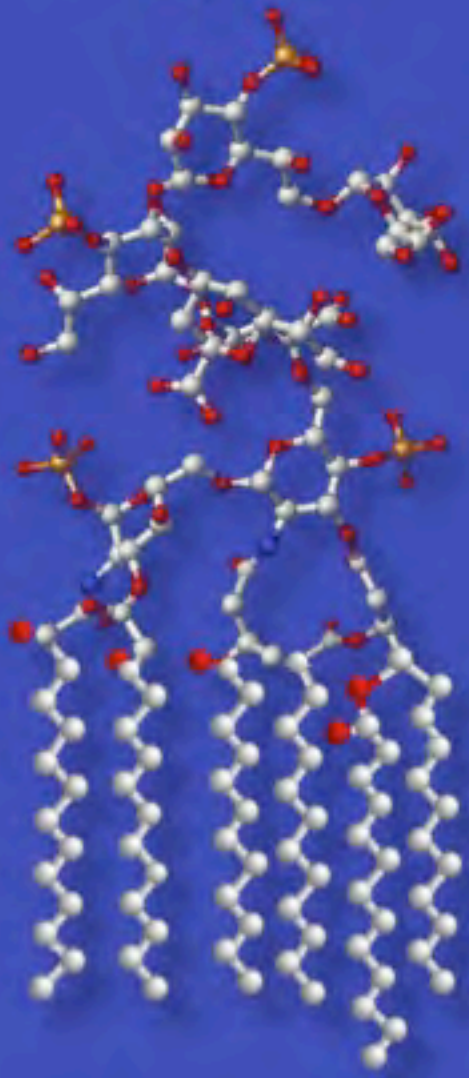


Gram negative bacteria
Eschericia coli

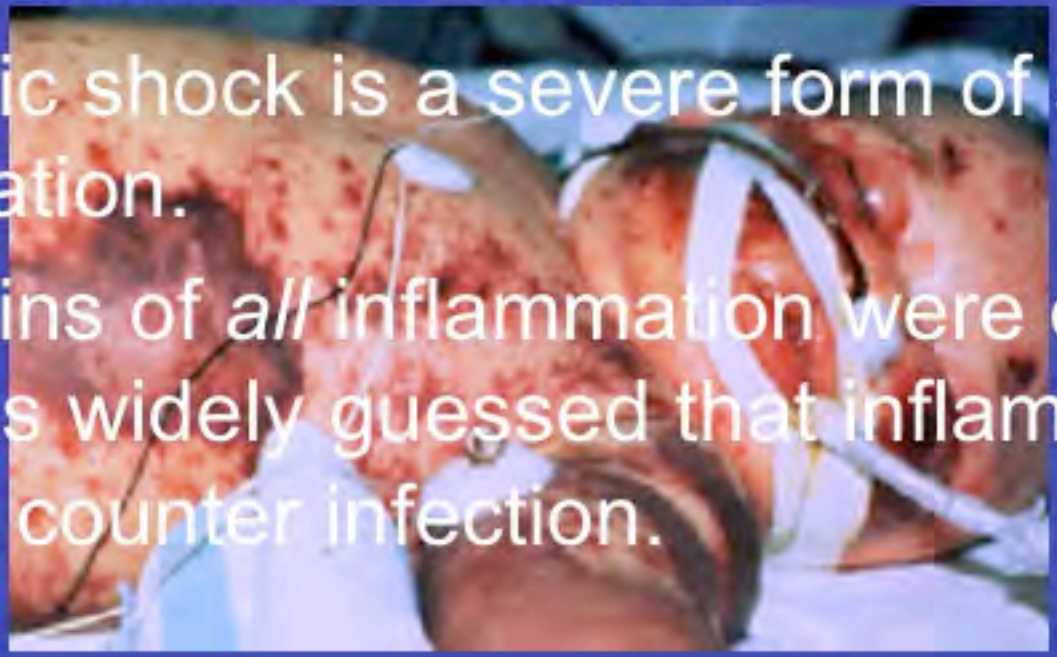


LPS



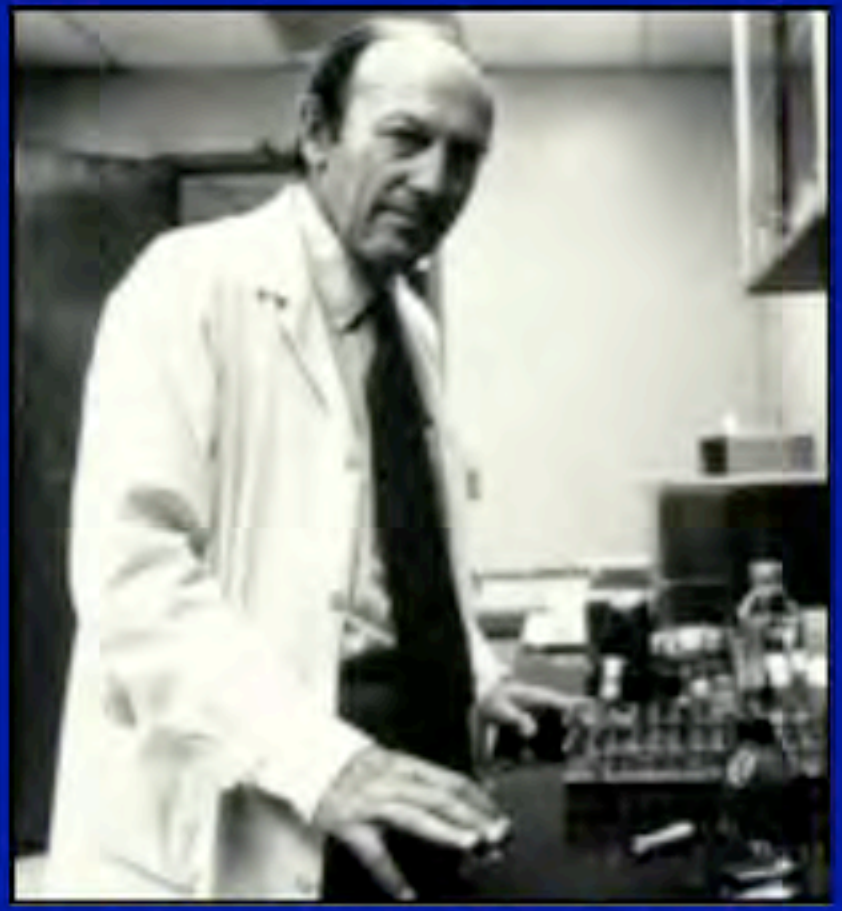
Richard Pfeiffer was nominated 33 times to receive the Nobel Prize in Physiology or Medicine.

- Hundreds of people die of endotoxin-induced shock every day, the result of severe Gram-negative bacterial infections.
- Endotoxic shock is a severe form of systemic inflammation.
- The origins of *all* inflammation were obscure, but it was widely guessed that inflammation arose to counter infection.



Nearly 100 years went by from the discovery of endotoxin until its receptor was identified. This receptor was the key to understanding how the host becomes aware of infections.

I personally began to be interested in endotoxin almost 40 years ago.



In the summer of 1975 I worked in the lab of Abraham Braude, a pioneer in the clinical use of antibodies against LPS (1917-1984). There I first heard the word “endotoxin.”

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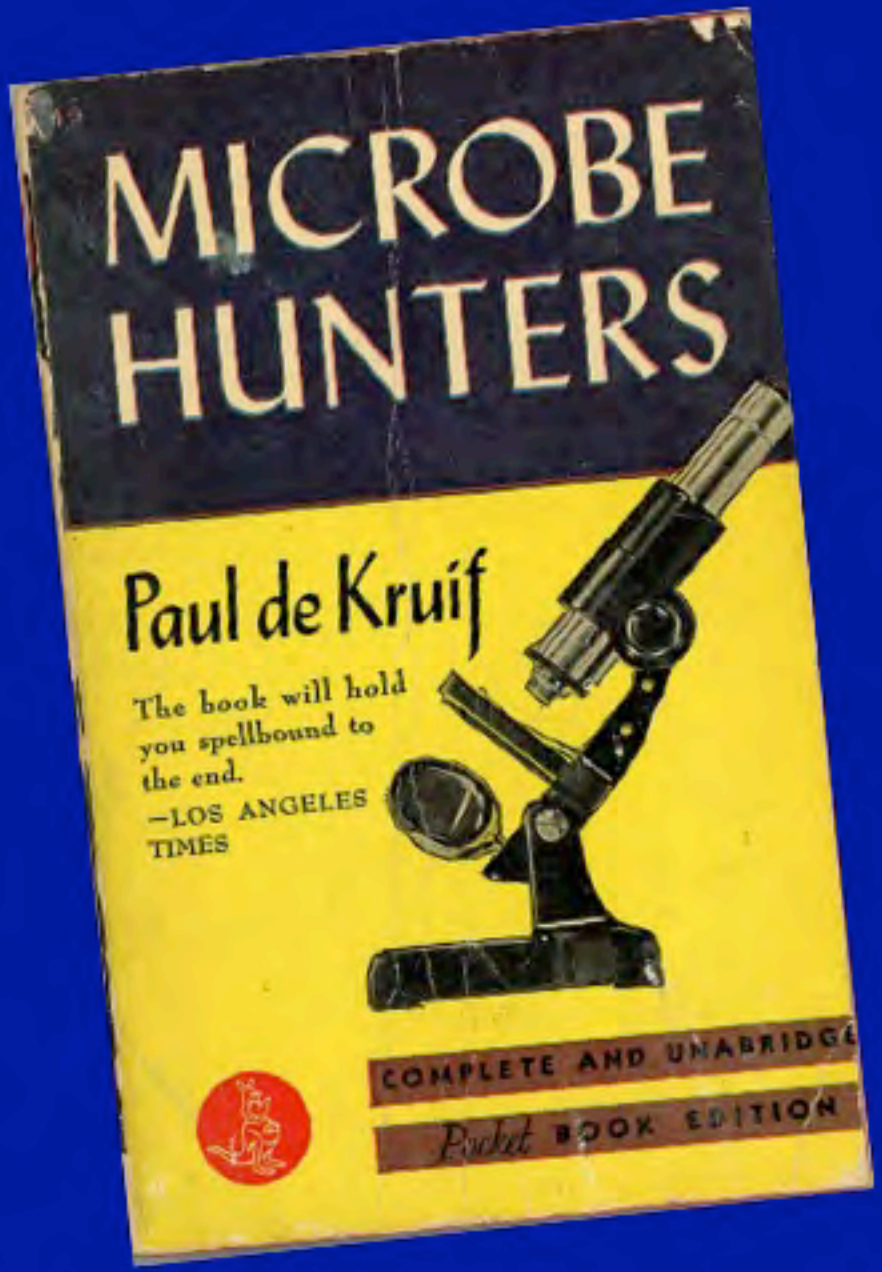

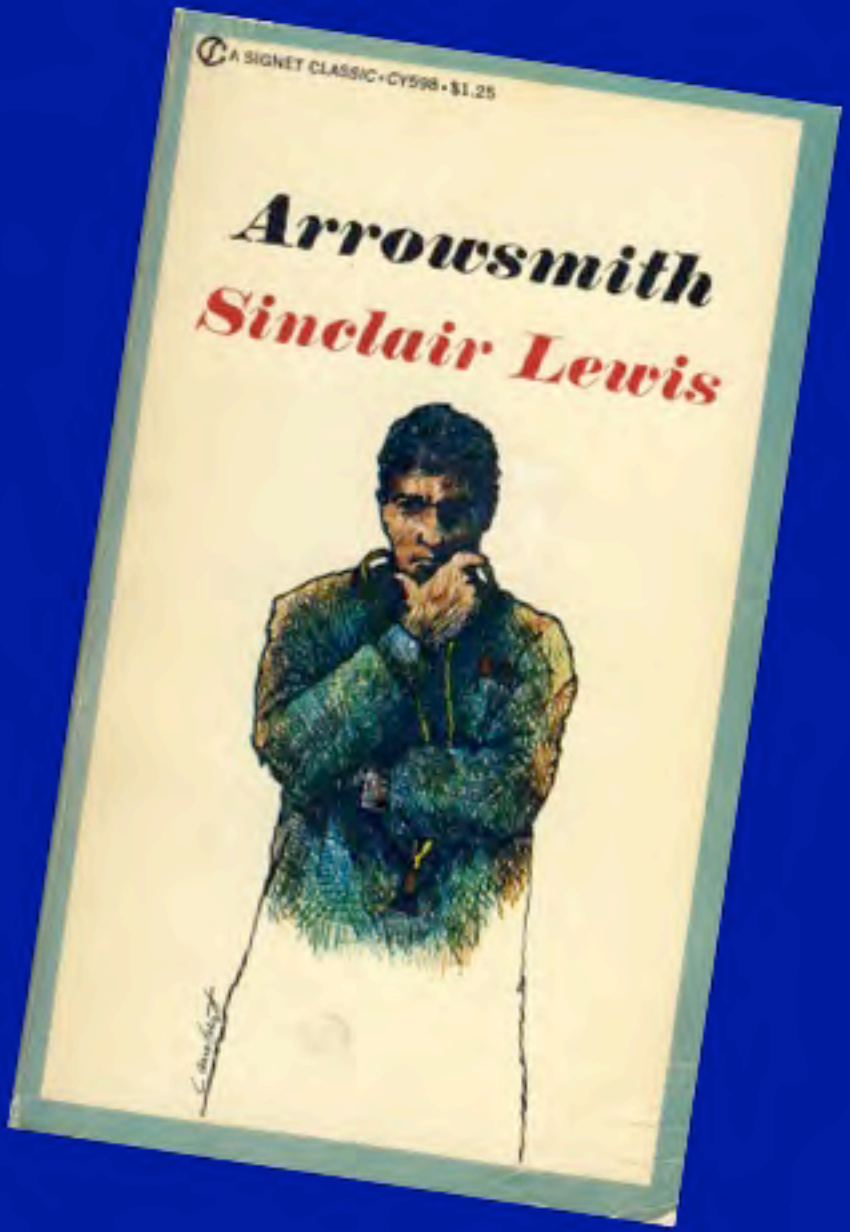

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These influences made me decide to go to medical school. Between 1977 and 1981, I went to the University of Chicago School of Medicine.

There and afterward, as an intern and resident (UT Southwestern), I saw many patients with severe infections, including gram negative septic shock, caused largely by endotoxin. I also saw patients with wasting (cachexia) due to chronic diseases, including both infection and cancer.

Chronic infections can cause wasting (TB, HIV, many others).

3T3-L1
pre-adipocytes

↑ Plasma triglyceride

Fat cells
(adipocytes)

↓ LPL, AcCoAC, FAS

Tumors

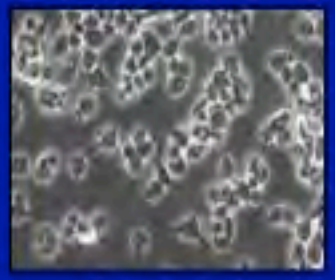
?

Cachectin

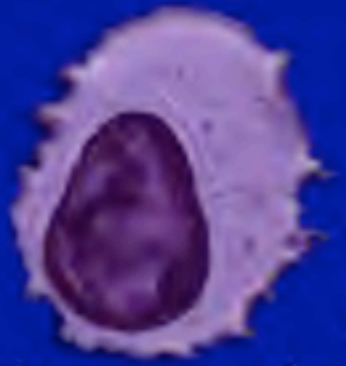
?

Other
tissue

Cachectin: a postulated mediator of wasting (cachexia) in chronic disease



RAW 264.7
macrophages



Immune cells
(macrophages)

Endotoxin

Trypanosome factors

Tumor factors

Infection

Malignancy

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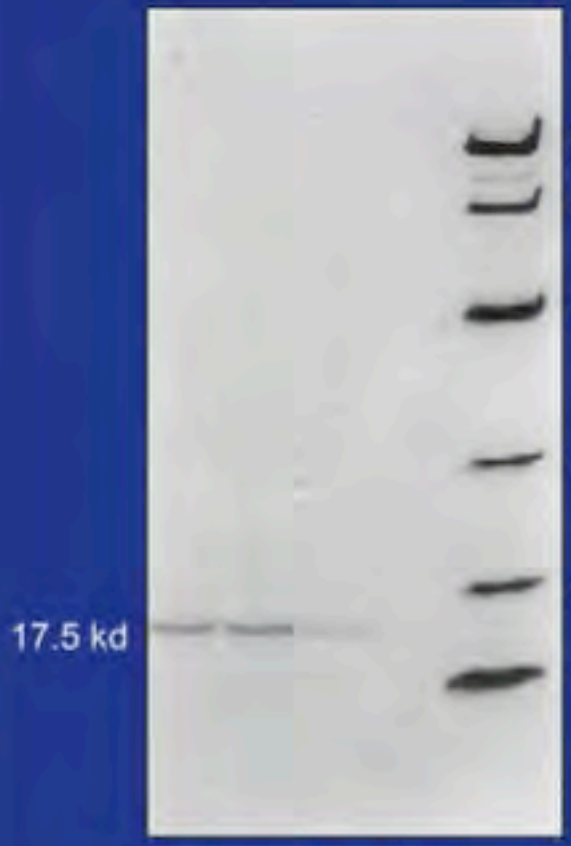
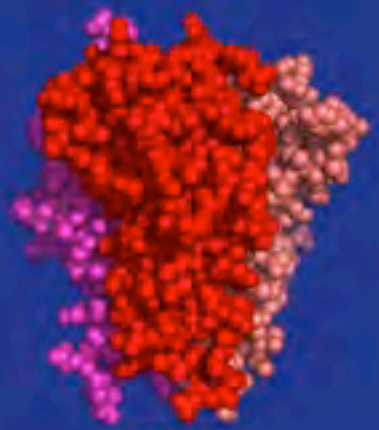
1983





Cachectin = Mouse tumor necrosis factor

(mouse CACH)

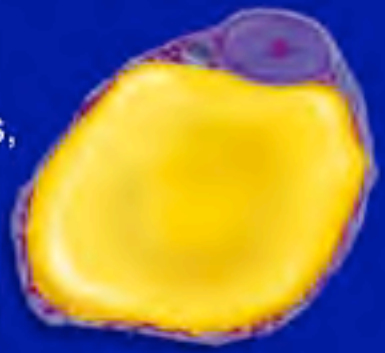
H_2N LEU-ARG-SER-SER-SER-GLU-ASN-SER-SER-ASP-PRO-PRO-VAL-ALA- ? -VAL-VAL-ALA-ASN...

H_2N VAL-ARG-SER-SER-SER-ARG-THR-PRO-SER-ASP-LYS-PRO-VAL-ALA-HIS-VAL-VAL-ALA-ASN...

(human TNF)

1 µg of cachectin had 10^8 U of TNF activity

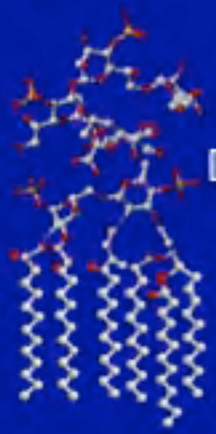
This raised the question: might TNF mediate *all* effects of LPS, including the lethal effect?



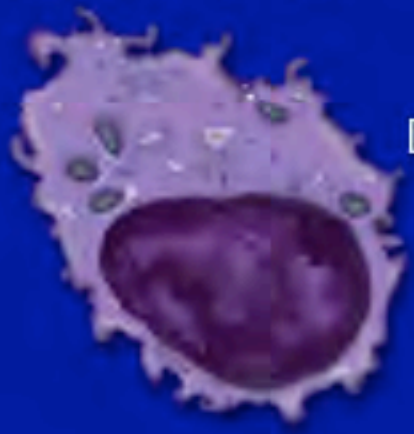
Triglyceride synthesis, LPL, FAS

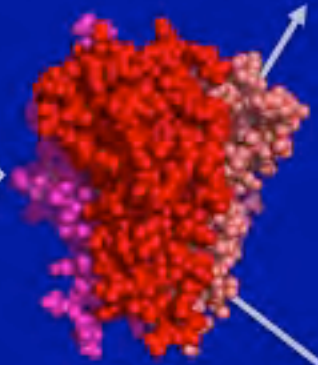
AcCoA carboxylase, glycerol release

LPS



MACROPHAGE

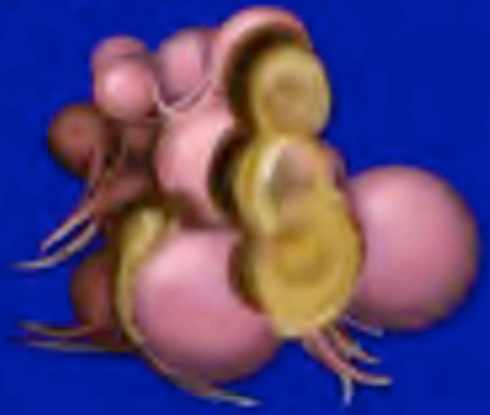




FAT

TNF

TUMOR



Tumor necrosis and cytolysis

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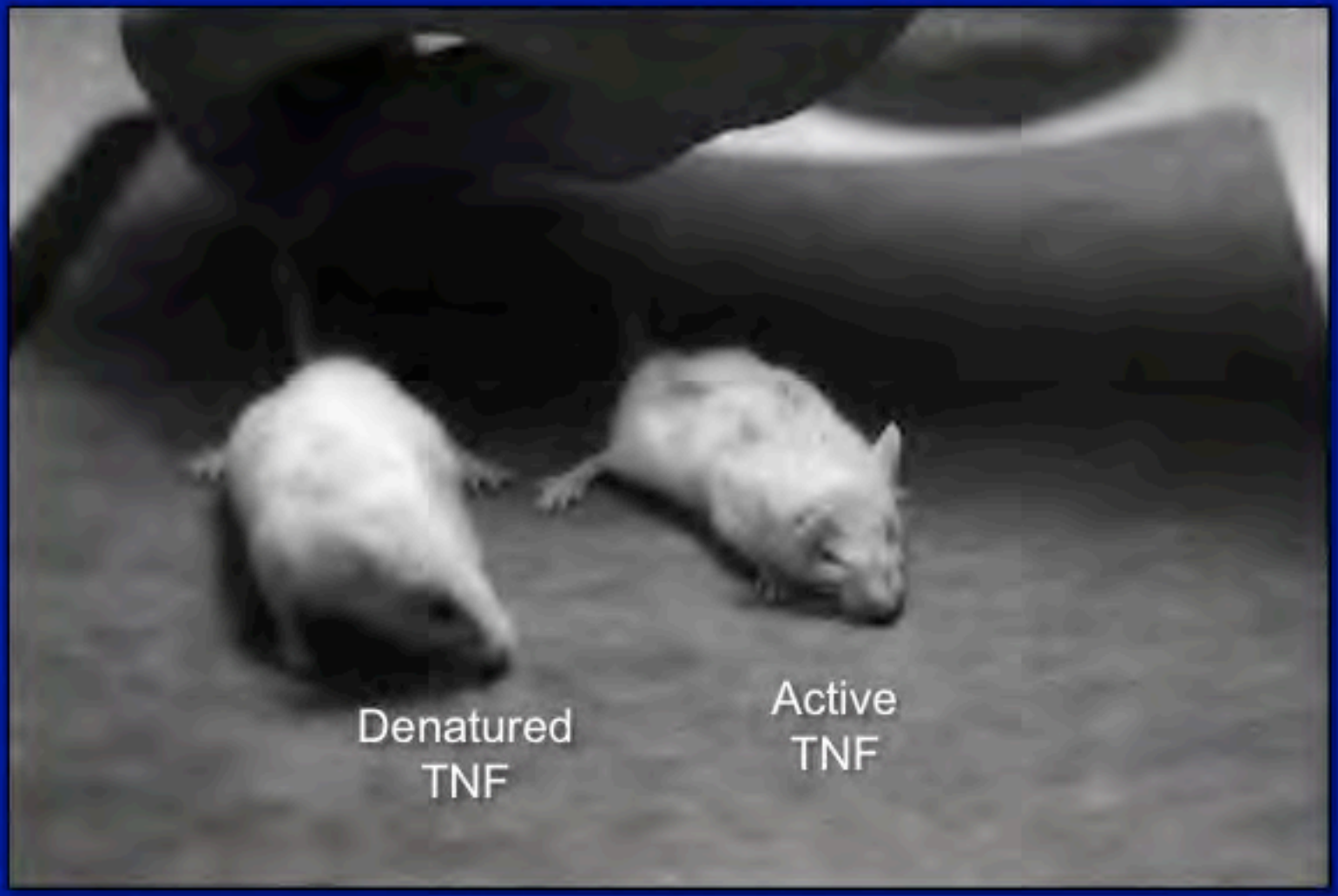
Purified TNF mimics LPS in its toxicity



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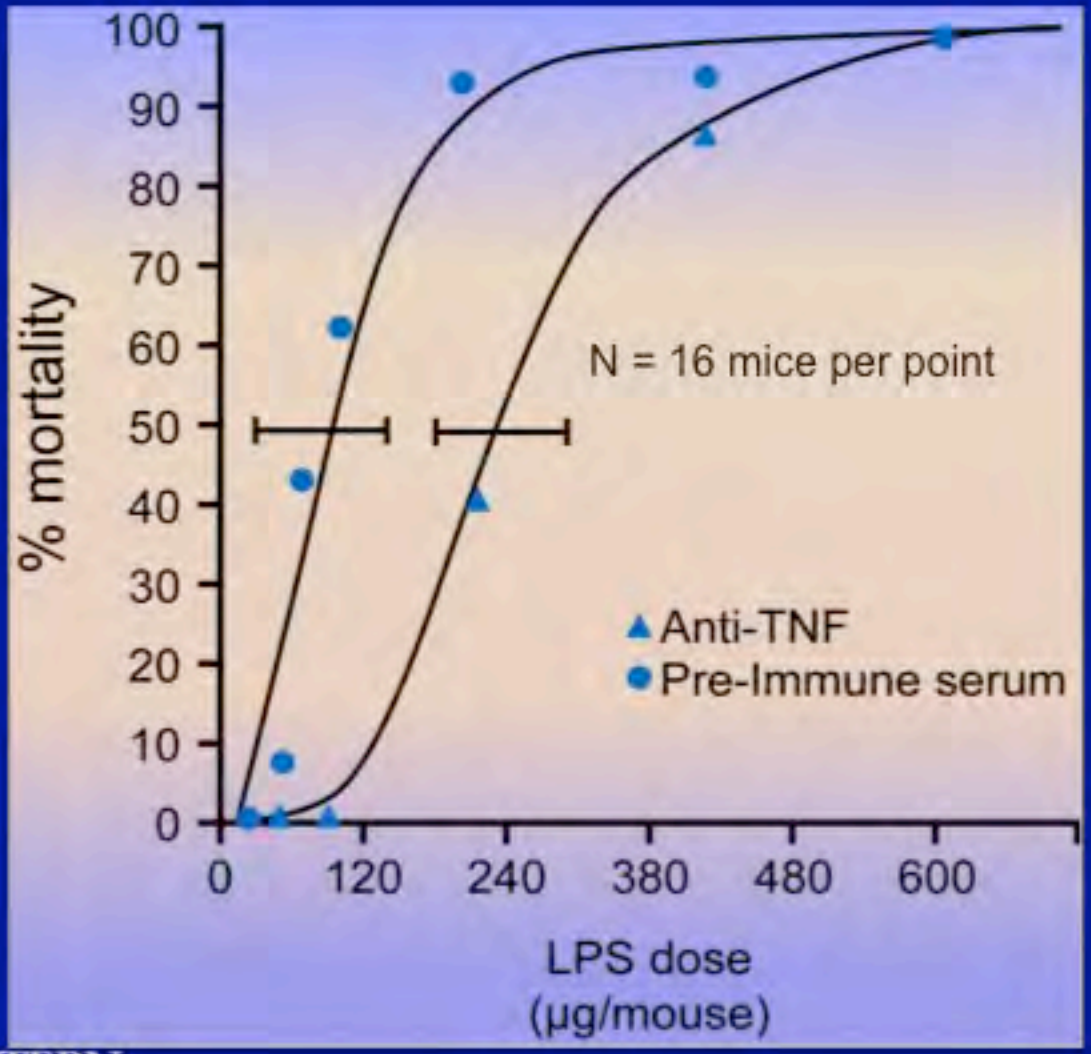
1984

Anti-TNF antibody blocks the lethal effect of LPS



1985

The importance of the TNF work was as follows…

In understanding what causes damage during infection:

- We had moved beyond putrefaction, caused by miasmas or contagions.
- We had moved beyond bacteria.
- We had moved beyond substances made by bacteria, such as LPS.
- We now knew of a protein mediator of injury, synthesized by the host.
- This protein could be the ideal endpoint to follow in monitoring the detection of microbes by the host.

In 1986, I returned to UT Southwestern, and to HHMI, to study TNF further…

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I had two major objectives in mind.

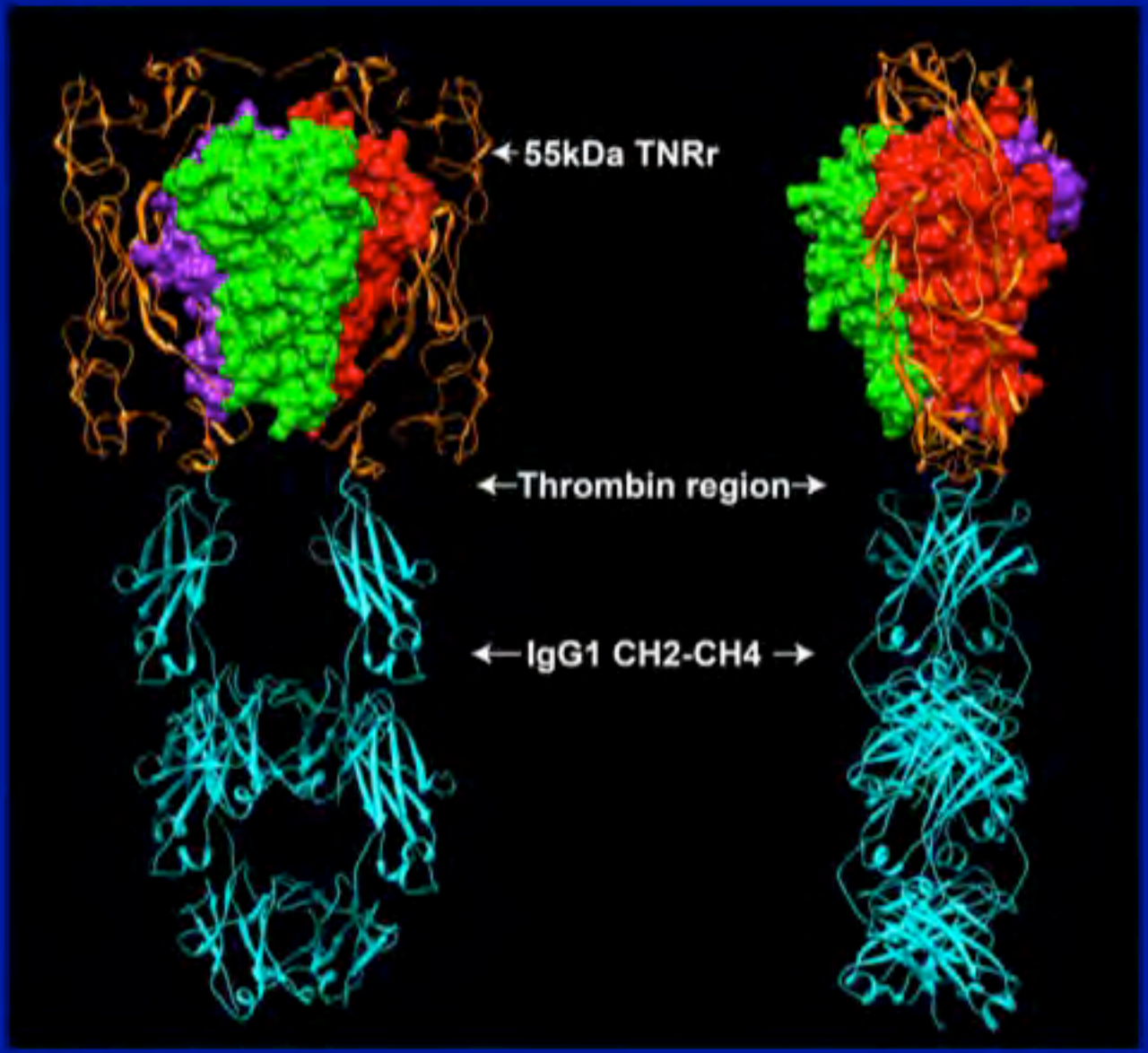
1. To block TNF activity (both to see what effects that would have on host resistance and to treat inflammatory diseases).

2. To study how TNF synthesis was regulated.

The TNF receptors were isolated and cloned in 1990 by David Wallach (Israel) and by David Goeddel (USA).

Karsten Peppel, David Crawford, and I hooked the receptor ectodomains to antibody heavy chains to make highly effective, specific, and stable inhibitors of TNF.

Later, as Enbrel, these inhibitors were used to treat rheumatoid arthritis and other inflammatory diseases.



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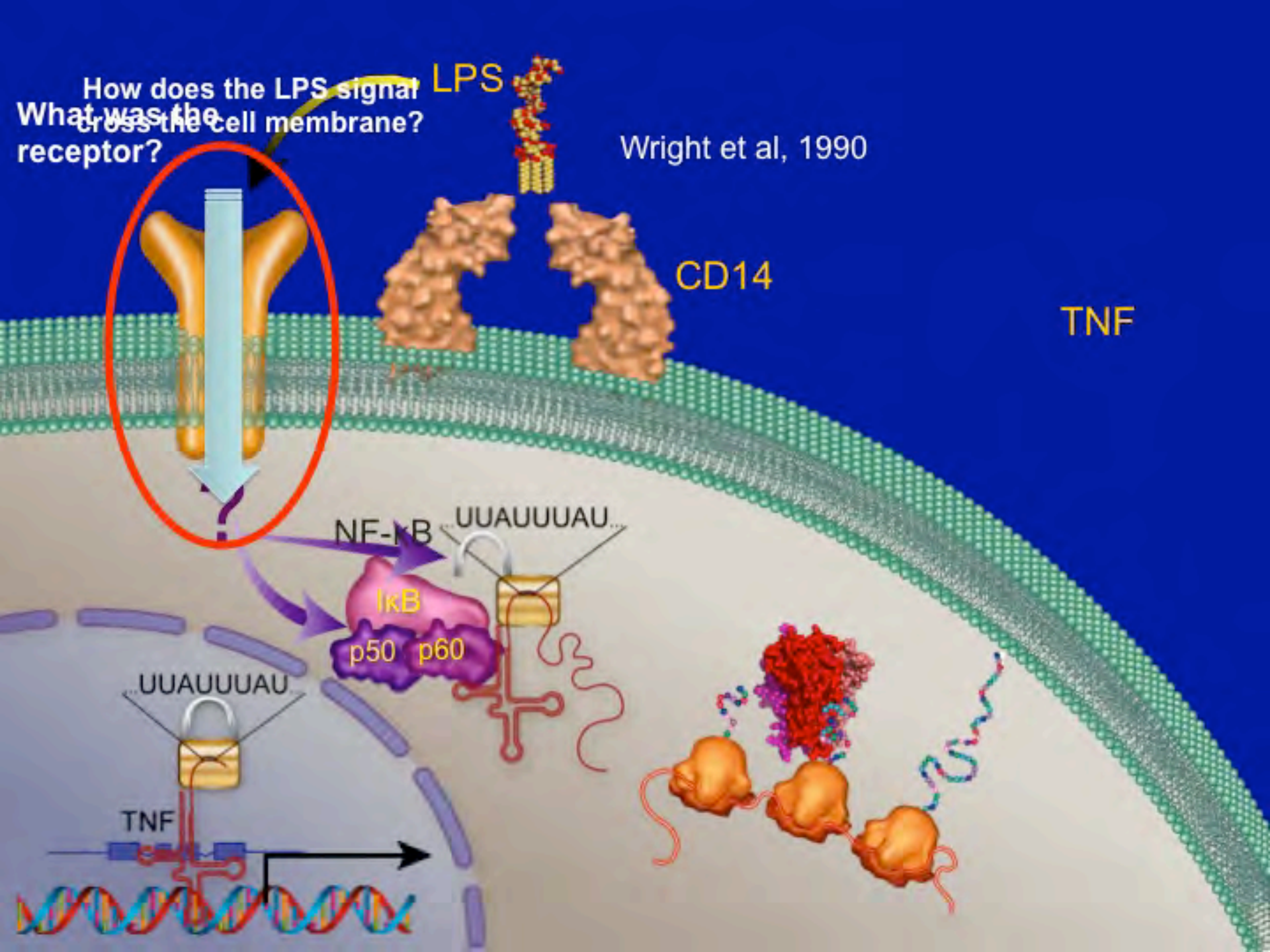
Peppel et al, *JEM:* 174(6):1483-9, 1991

The second problem we worked on was much more challenging …

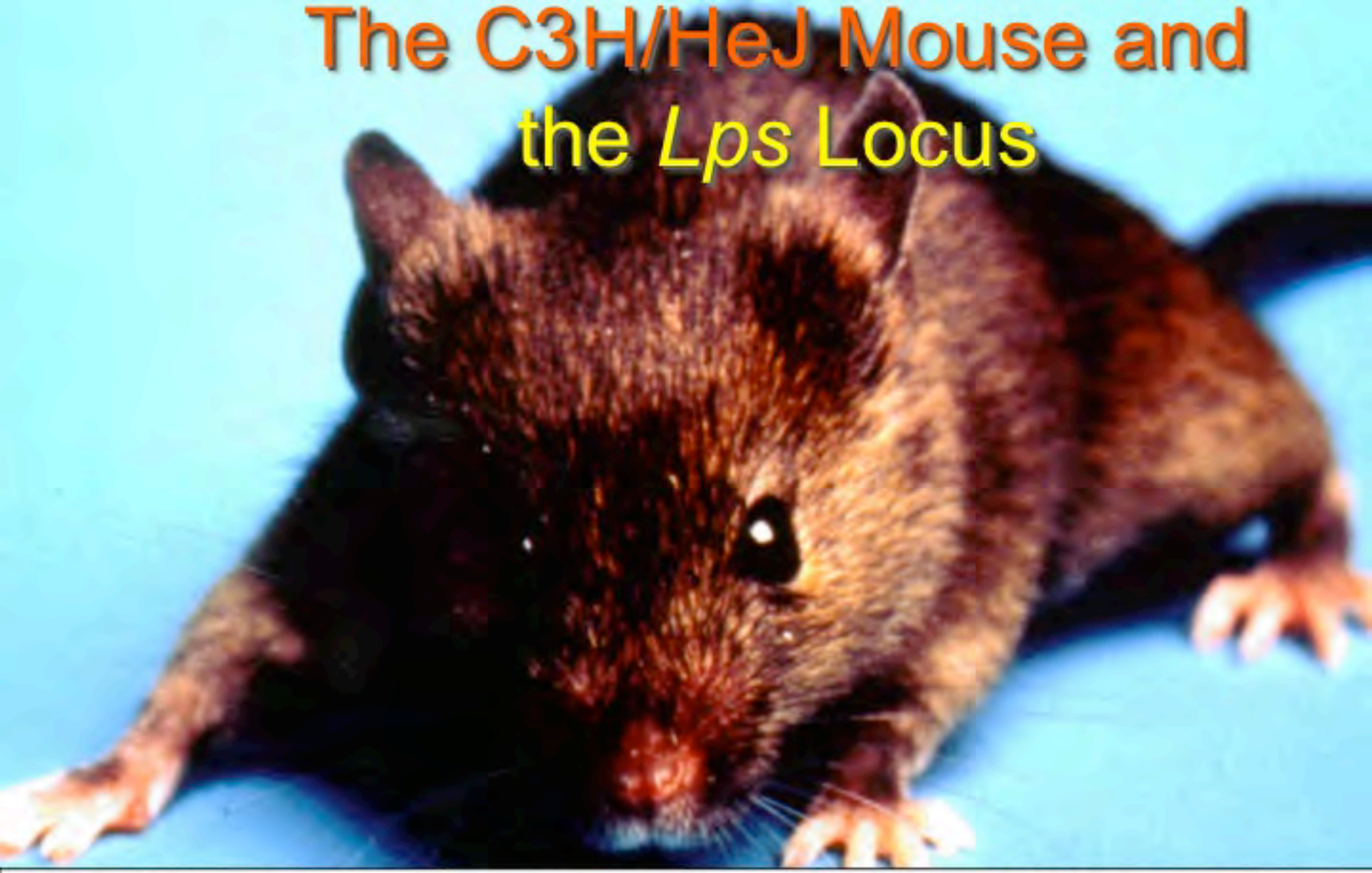
In studying the regulation of TNF biosynthesis, we were inquiring into the age-old problem of how endotoxin (LPS) was sensed by the host. What was the receptor for LPS?


How does the LPS signal cross the cell membrane?
What was the receptor?
LPS
Wright et al, 1990
CD14
TNF
NF-κB
UUAUUUAU
IκB
p50
p60
UUAUUUAU
TNF

The answer to the question lay in two peculiar substrains of mice that wouldn't respond to LPS

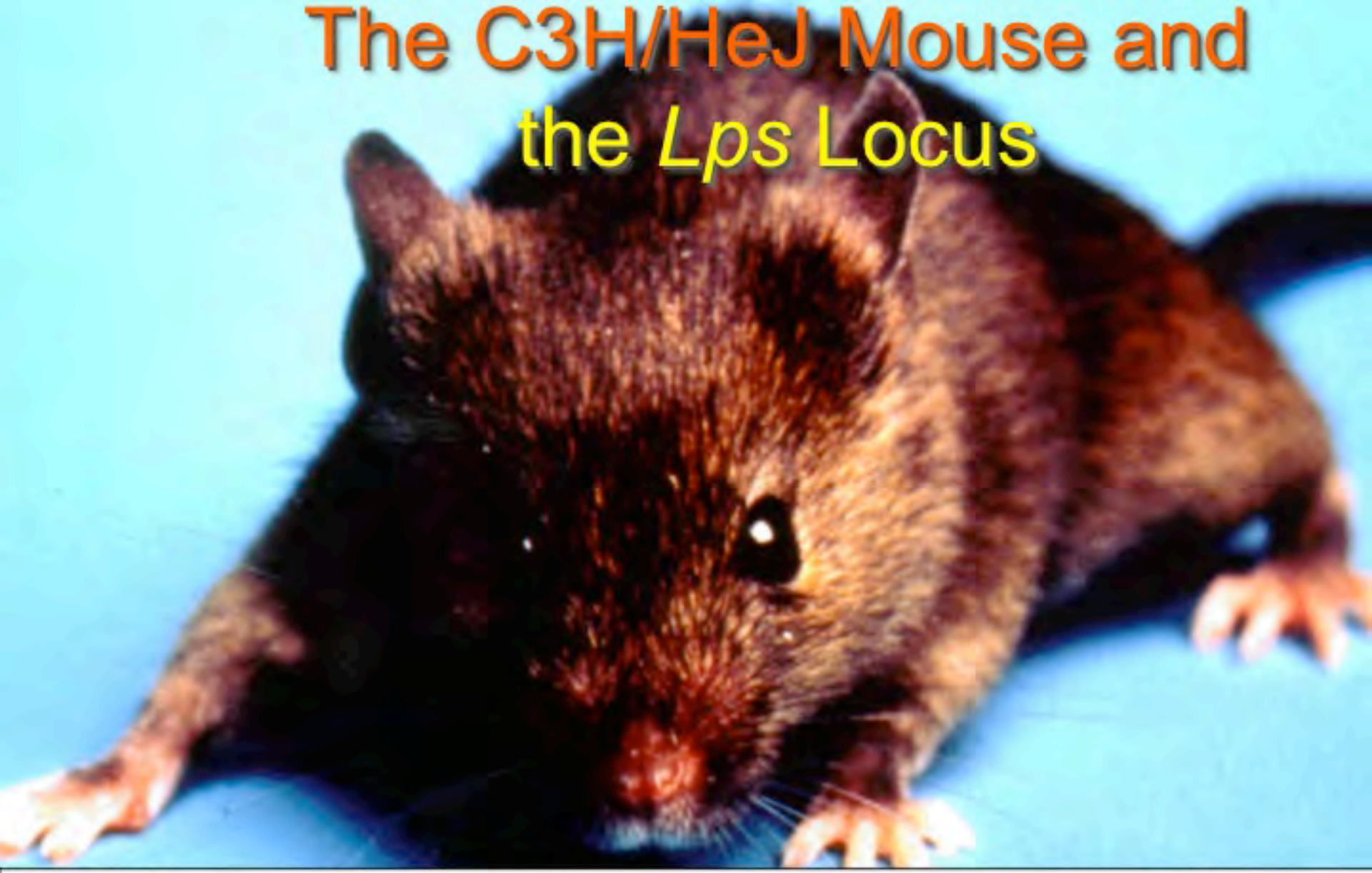
- The very existence of such mice suggested that LPS was sensed in a specific way.
- All effects of LPS were dependent on a single, specific protein.
- But which protein?

The C3H/HeJ Mouse and the *Lps* Locus



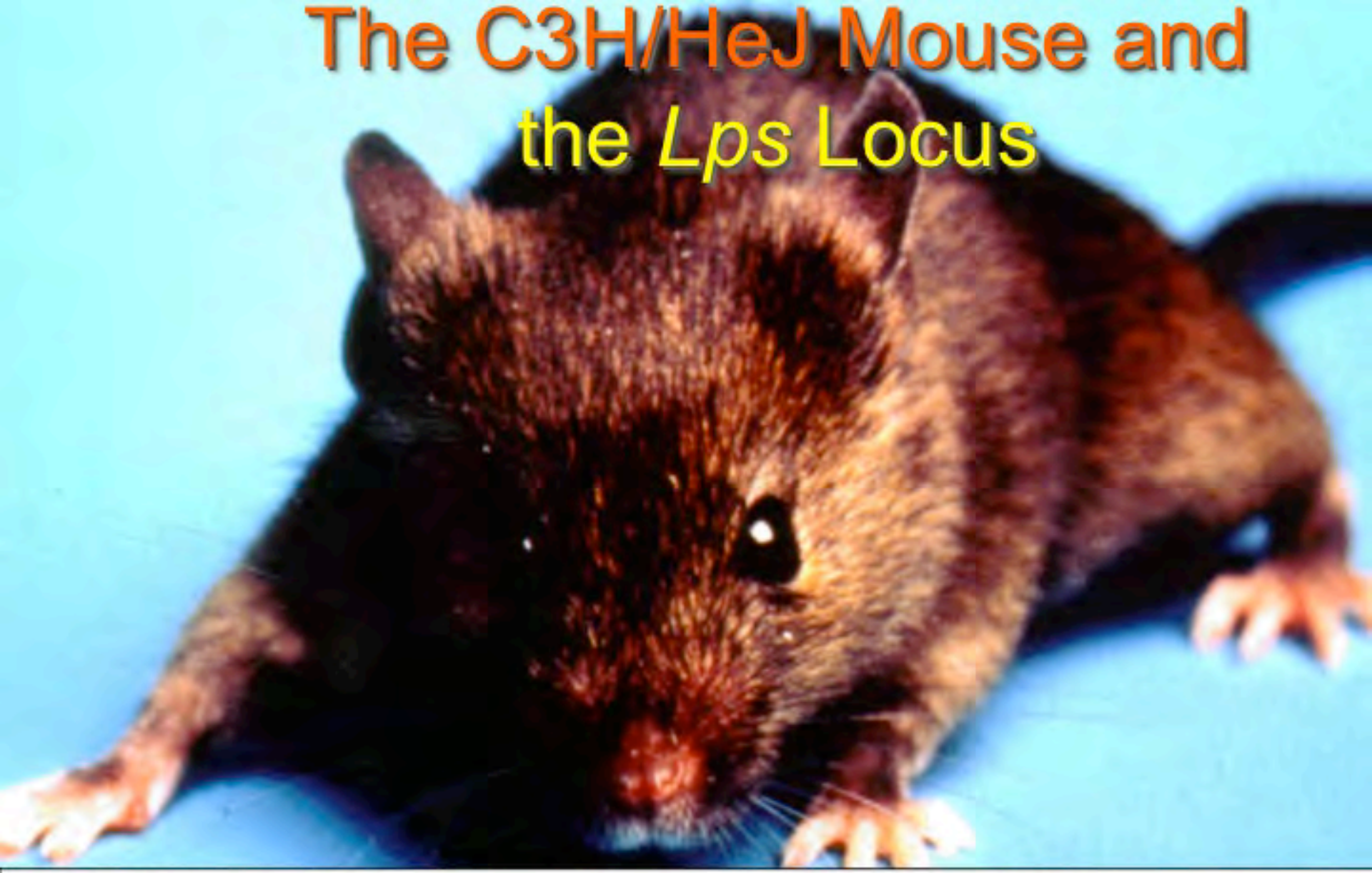
- Resistant to LPS (Heppner and Weiss, 1965), but highly susceptible to Salmonella (O'brien, 1980)

The C3H/HeJ Mouse and the *Lps* Locus



- Fail to make a cytokine response to LPS (for example, no TNF), but *only* to LPS.

The C3H/HeJ Mouse and the *Lps* Locus



- *Lps* mapped to Chr. 4 between *Mup1* and *Polysyndactyly* loci by Watson et al. in 1978.

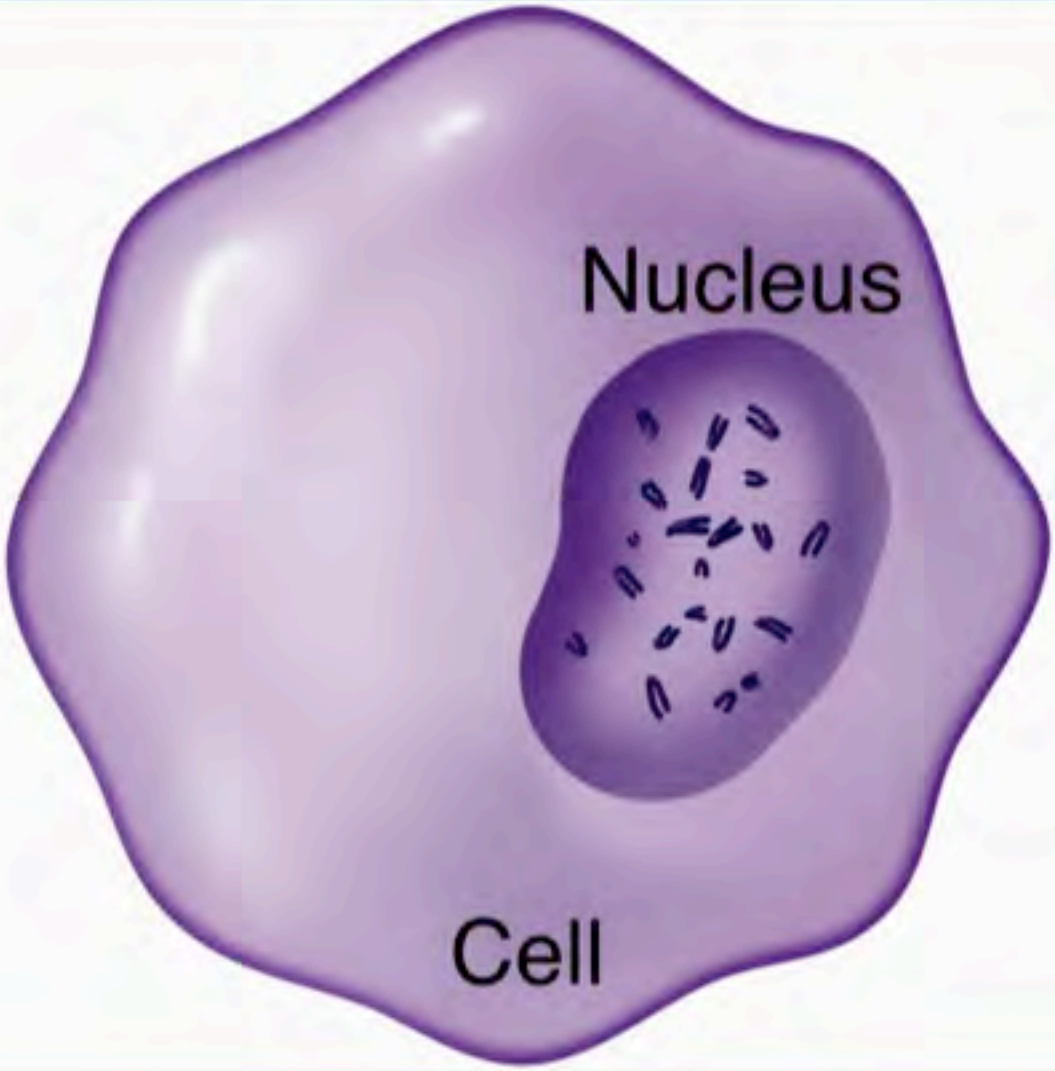
The C57BL/10ScCr Mouse and the *Lps* Locus

- The same gene was affected in the LPS-refractory C57BL/10ScCr strain (Coutinho and Meo,1978).

Early in 1993, my group began to search for the gene that was defective in these mice by positional cloning.

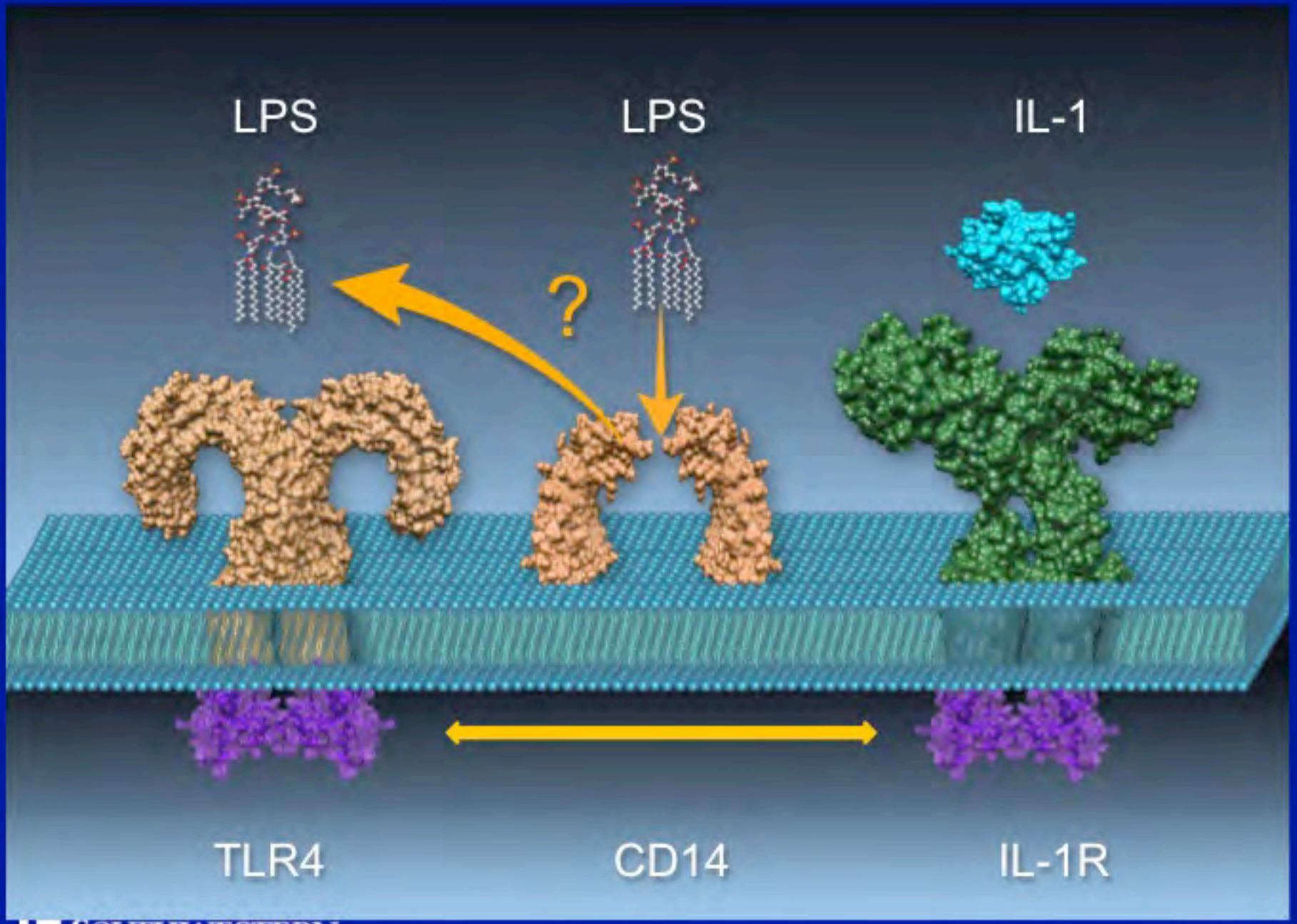
- We soon worked on this to the exclusion of all other projects in the laboratory.
- The search for the gene lasted five years, and became an all-consuming obsession.

There are 2.7 billion base pairs in the mouse genome



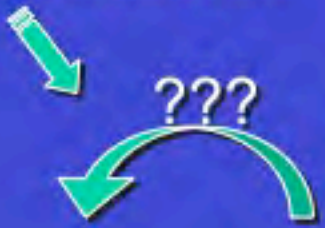
By August 1998, almost all of the critical region had been explored.

2.6 Mb


LPS
LPS
IL-1
?
TLR4
CD14
IL-1R

In *Drosophila*, the Dorsoventral Regulatory Cassette is also required for the Innate Immune Response to Fungal Infection

Fungal infection



???

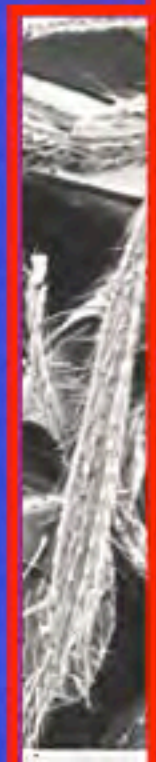
Spaetzle Pro-spaetzle

Toll

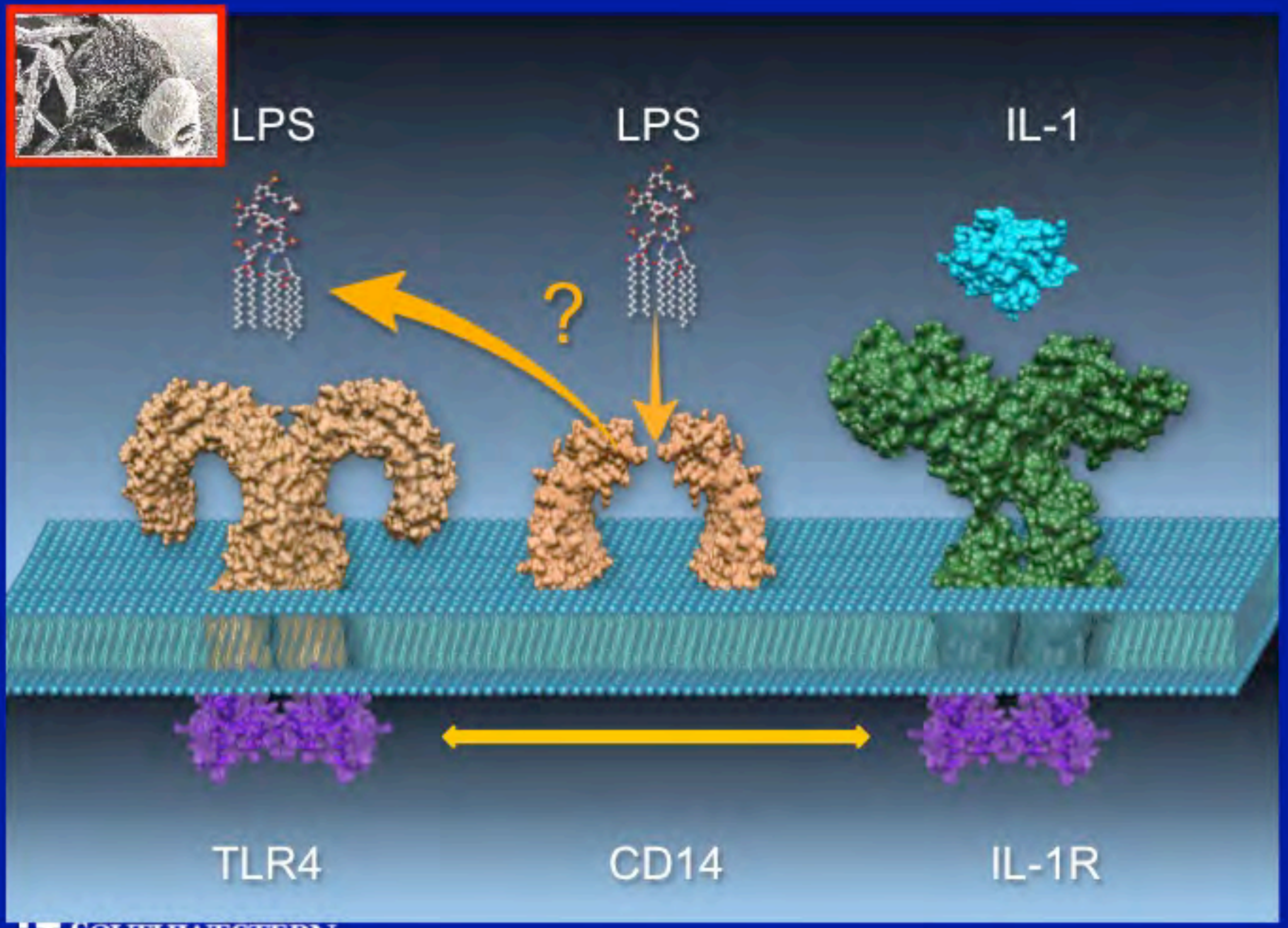
Tube, Pelle

Cactus, **Dif**

Drosomycin



Jules Hoffmann was interested in insect immunity, and worked in Strasbourg, FR

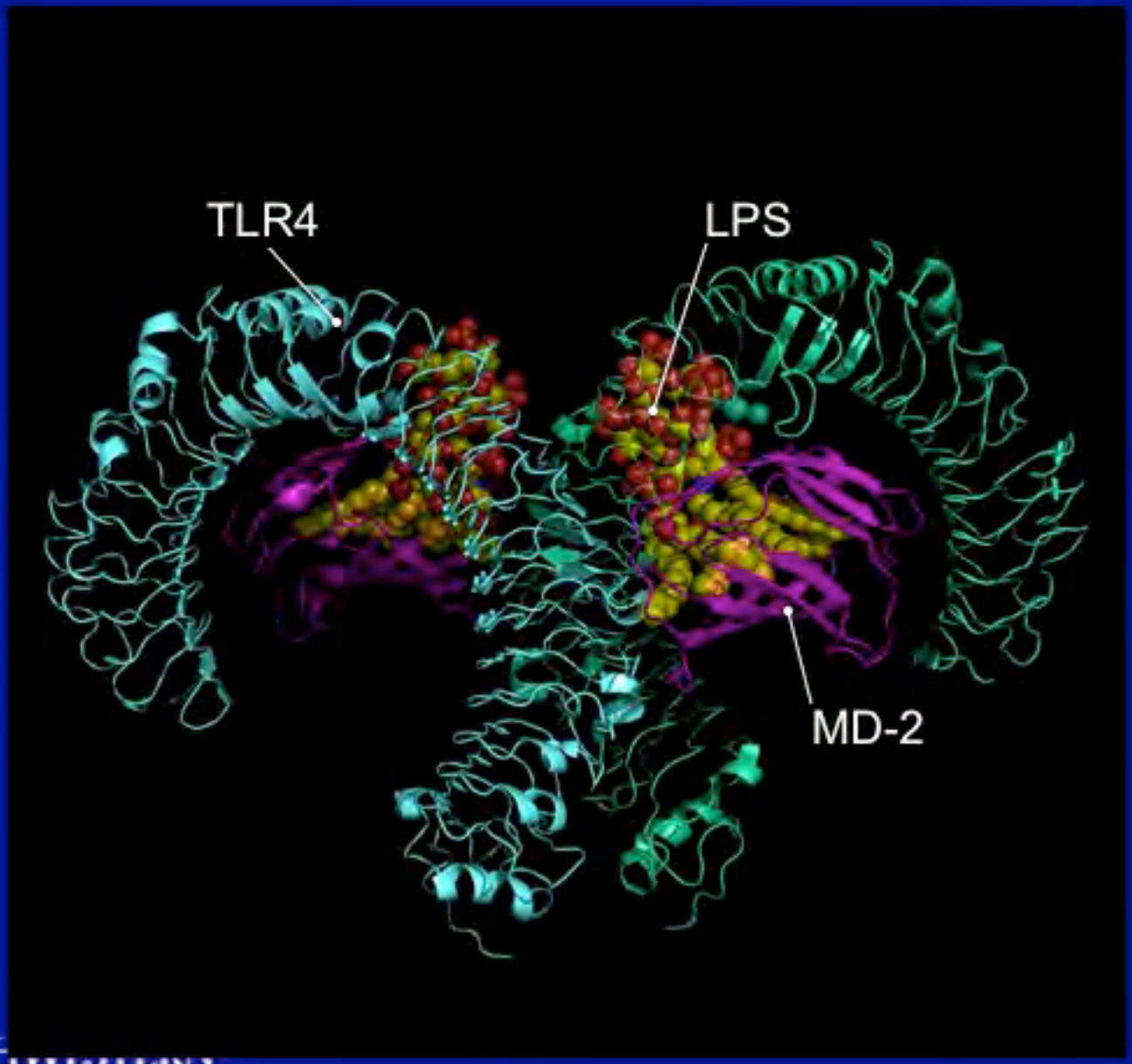

LPS
LPS
IL-1
?
TLR4
CD14
IL-1R

- In LPS-resistant mice, there were mutations that destroyed TLR4. In C3H/HeJ mice, the mutation was a single base change. In C57BL/10ScCr mice, the mutation was a large deletion. Finding these mutations was a magical moment: we knew we had found the LPS receptor, which had been wondered about for nearly 100 years.

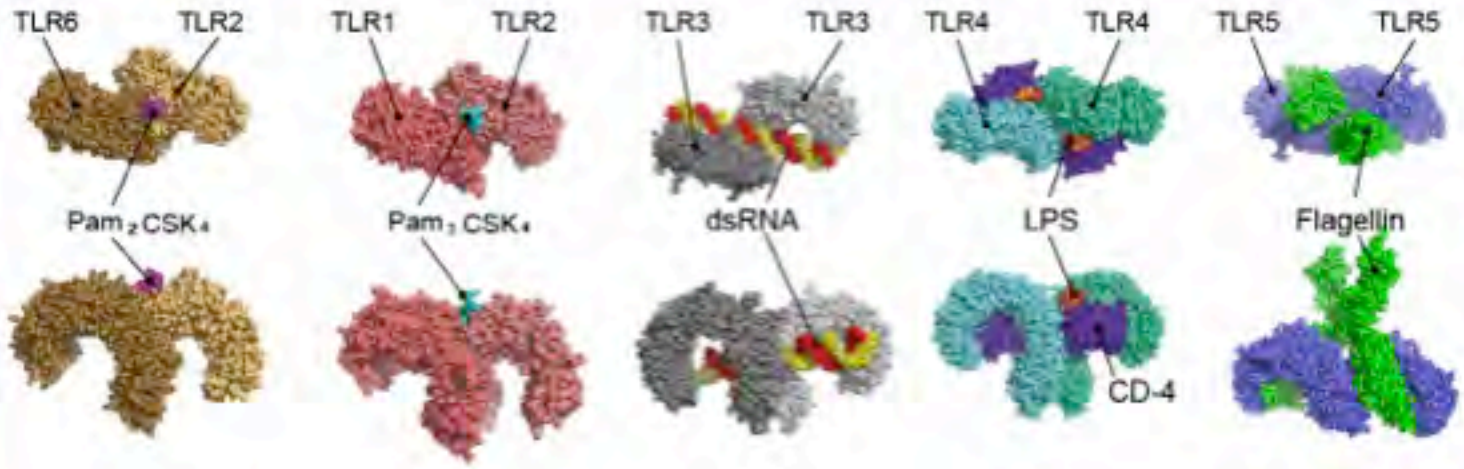
- We also deduced, based on genetic experiments, that LPS had direct, physical contact with TLR4.

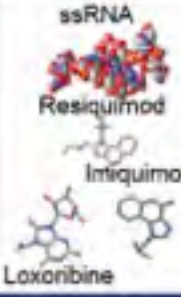
- Soon, it was established by others that MD-2, a small secreted protein, was also part of the LPS receptor complex (Kensuke Miyake and colleagues).

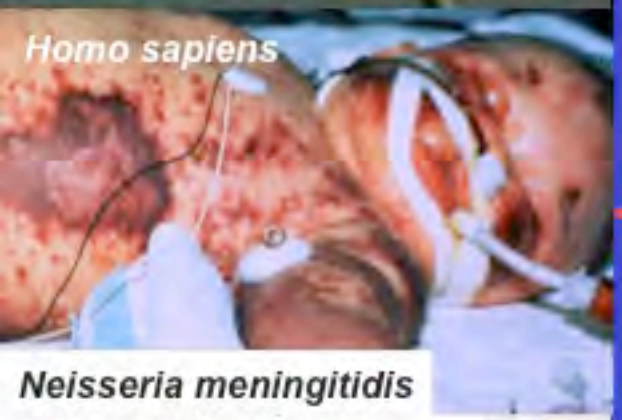
- About ten years later, the structure of the entire complex was solved (by Jie-Oh Lee and colleagues).


TLR4
LPS
MD-2

Now, the mode of binding of several ligands to TLRs is understood



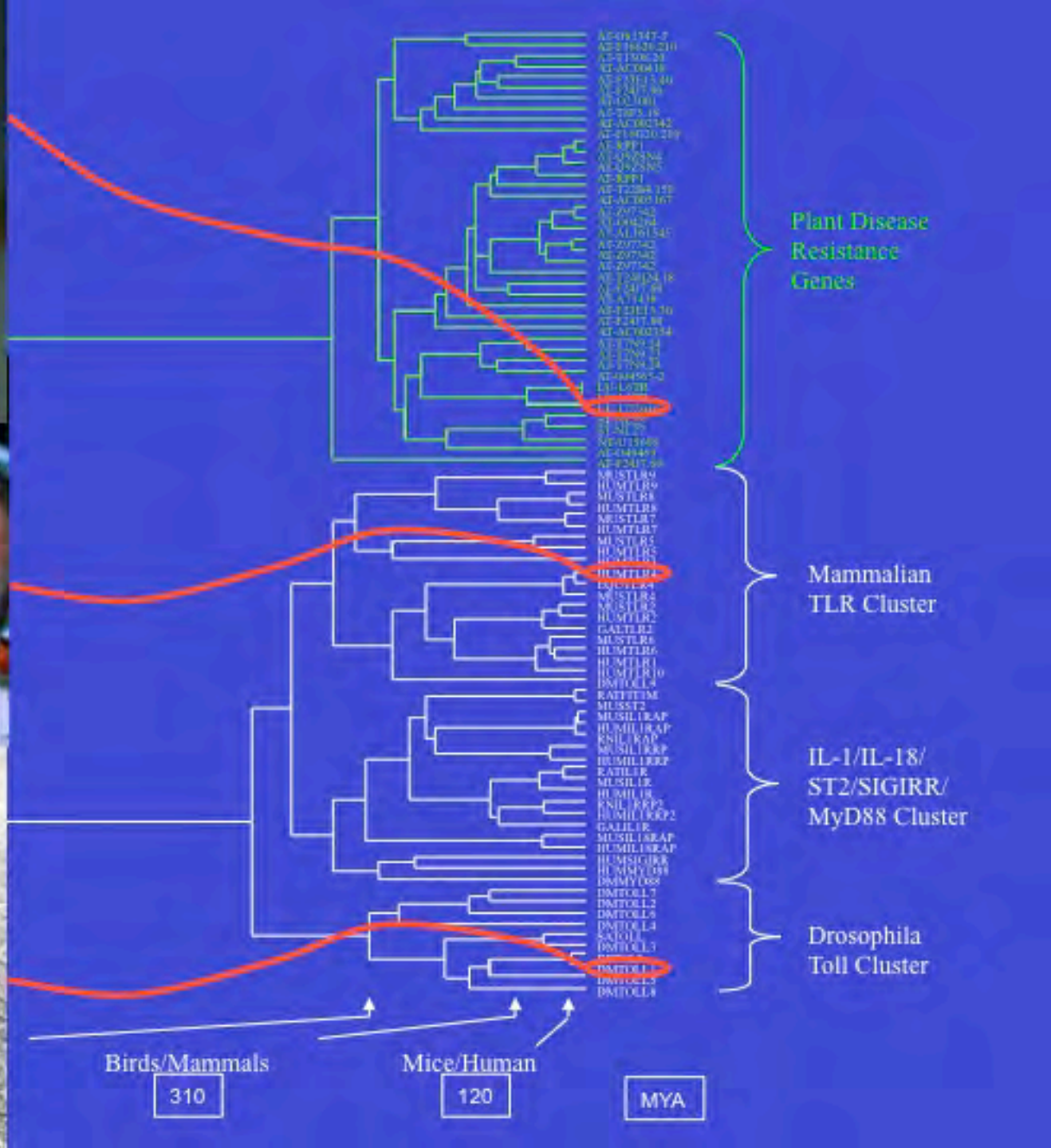
Receptor	TLR 1/2 or 2/6	TLR3	TLR4	TLR5	TLR7	TLR9
Ligands	Lipoproteins	Poly I:C, dsRNA	LPS	Flagellin	ssRNA	CpG-DNA
Source	Gram pos bacteria, fungi	Viruses	Gram neg bacteria	Bacterial flagellum	Viruses	Bacteria
Examples						

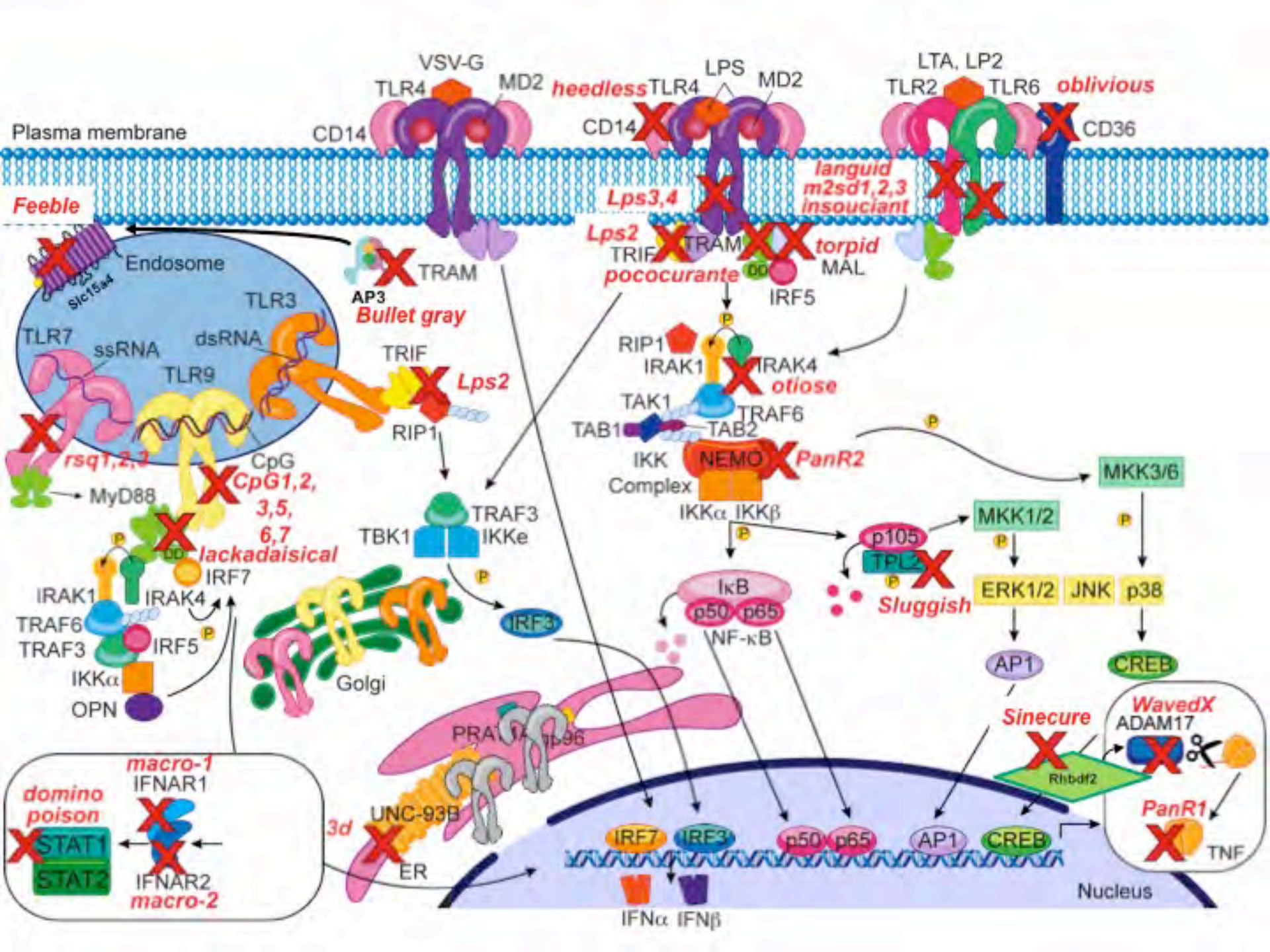


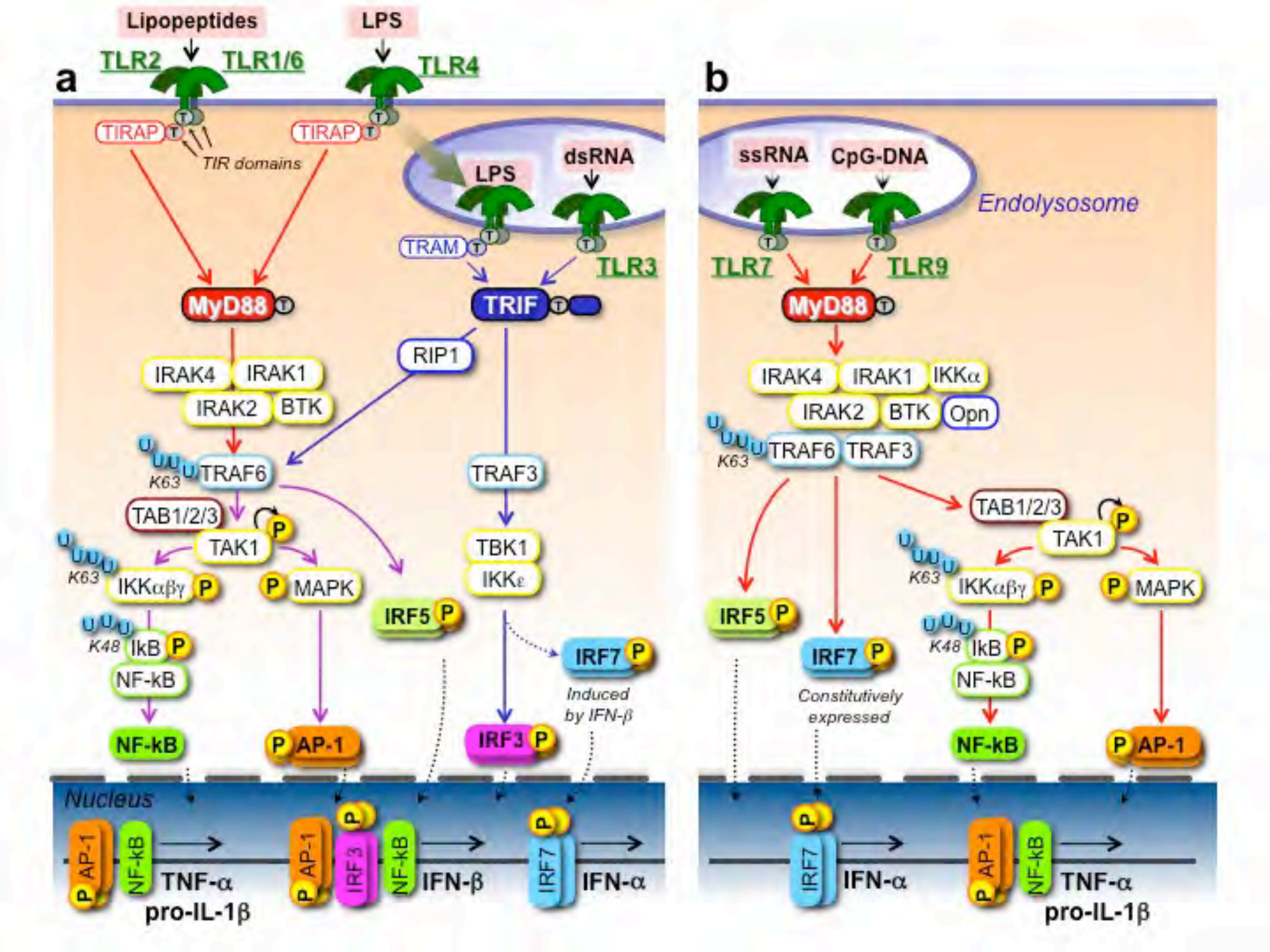
Neisseria meningitidis

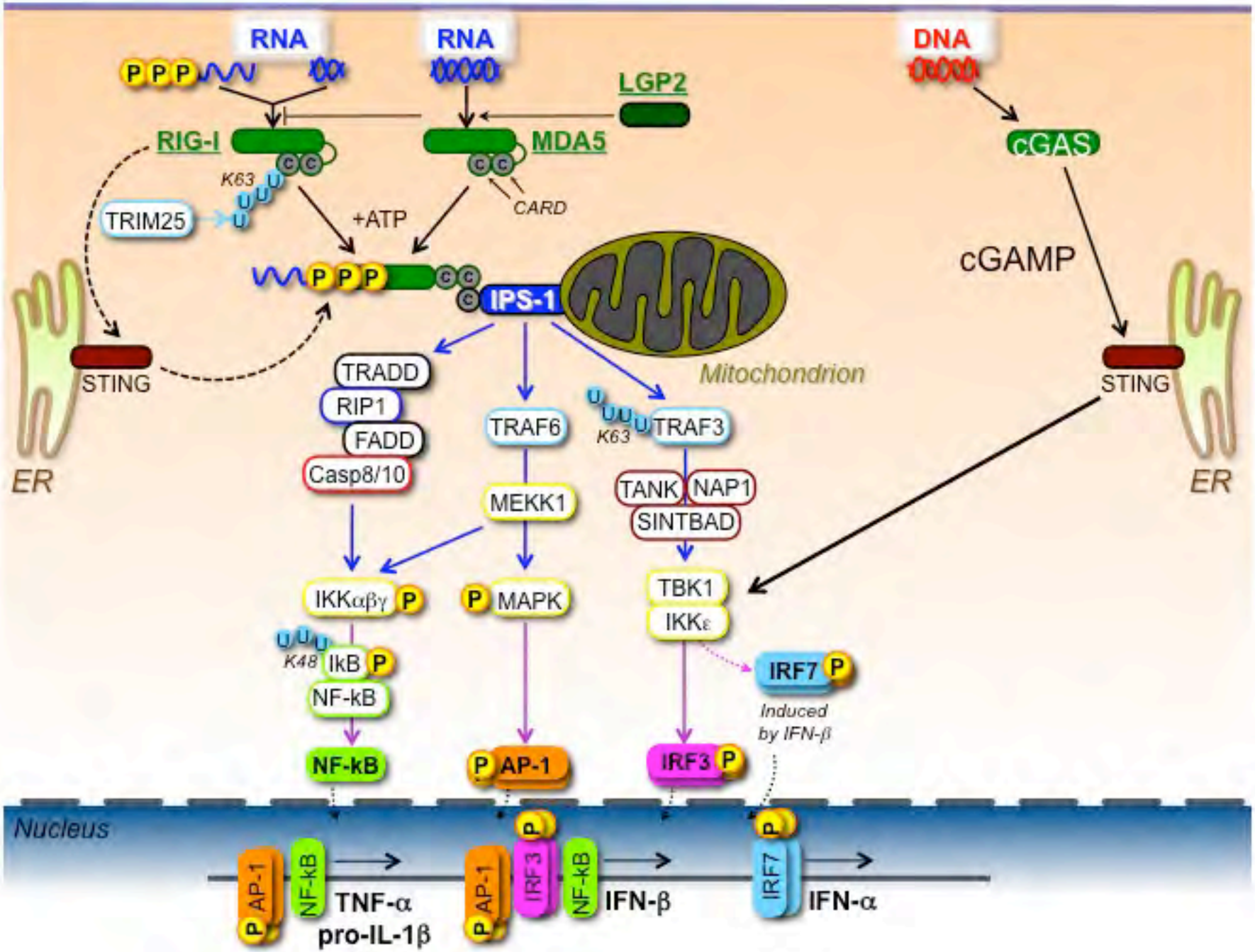
Drosophila melanogaster

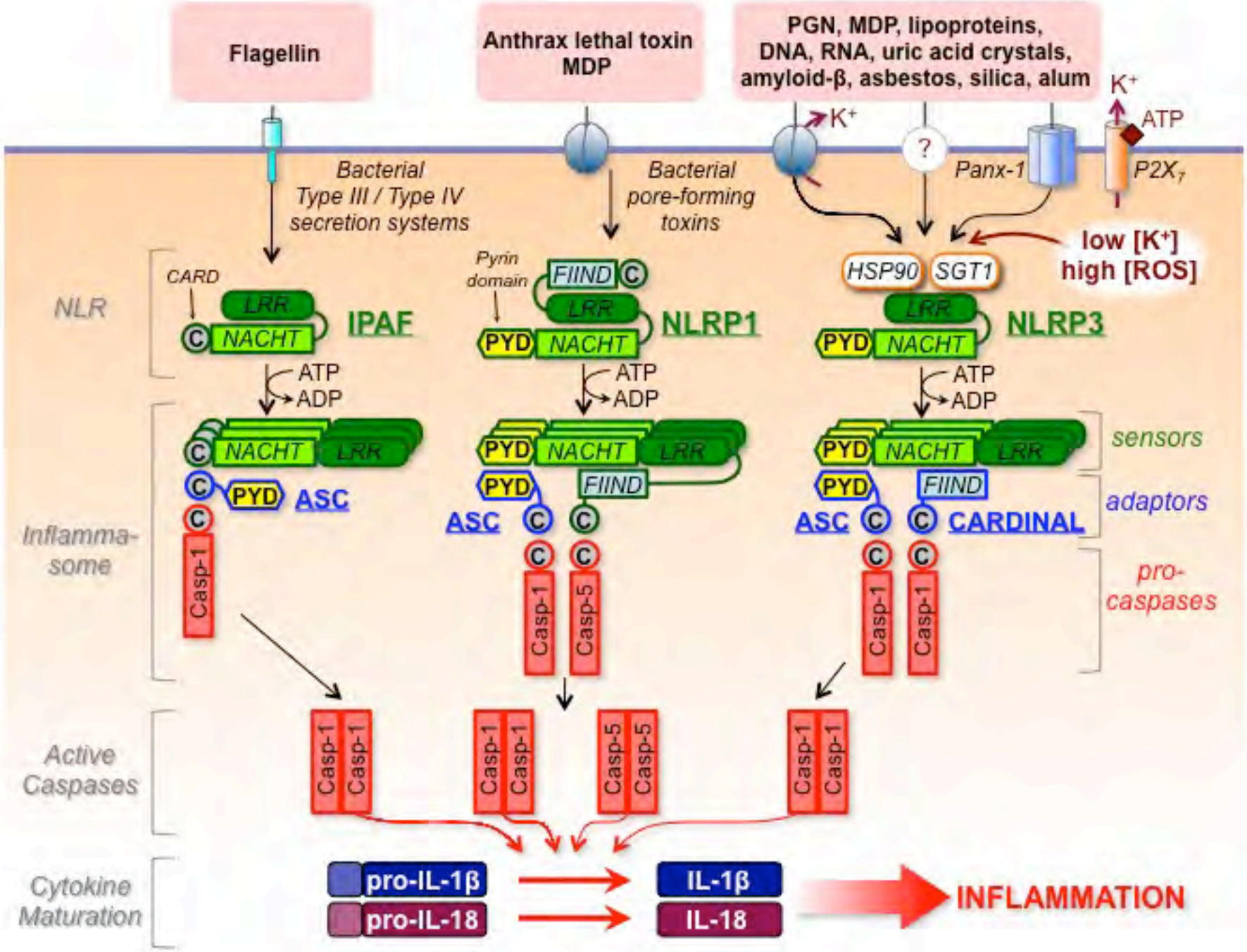
Aspergillus fumigatus

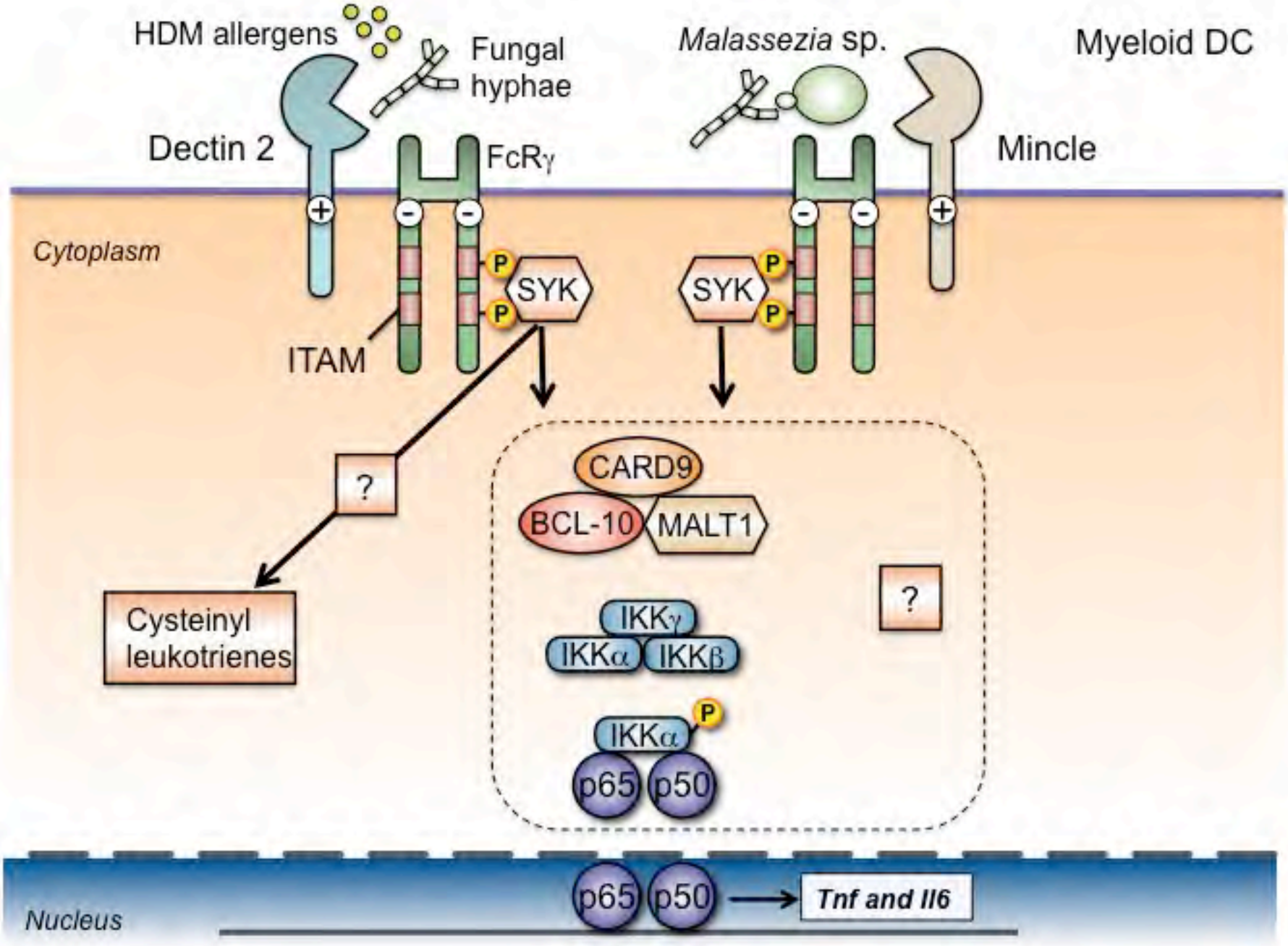



VSV-G
TLR4
MD2
CD14
heedless
LPS
LTA, LP2
TLR2
TLR6
oblivious
CD36
Plasma membrane
languid
m2sd1,2,3
insouciant
Feeble
Lps3,4
Lps2
TRIF
TRAM
pococurante
torpid
MAL
IRF5
Endosome
Slc15a4
AP3
Bullet gray
TLR3
TLR7
ssRNA
dsRNA
TLR9
RIP1
IRAK1
IRAK4
otiose
TAK1
TRAF6
TAB1
TAB2
IKK
Complex
NEMO
PanR2
rsq1,2,3
MyD88
CpG
CpG1,2,
3,5,
6,7
lackadaisical
IRF7
TRAF3
IKKe
TBK1
IRF3
MKK3/6
MKK1/2
p105
TPL2
Sluggish
ERK1/2
JNK
p38
IκB
p50
p65
NF-κB
AP1
CREB
IRAK1
IRAK4
TRAF6
TRAF3
IRF5
IKKα
OPN
Golgi
Sinecure
WavedX
ADAM17
Rhbdf2
PanR1
TNF
macro-1
IFNAR1
domino
poison
STAT1
STAT2
IFNAR2
macro-2
3d
UNC-93B
ER
IRF7
IRF3
p50
p65
AP1
CREB
Nucleus
IFNα IFNβ


a
Lipopeptides
LPS
TLR2
TLR1/6
TLR4
TIRAP
TIRAP
TIR domains
LPS
dsRNA
TRAM
TLR3
MyD88
TRIF
RIP1
IRAK4
IRAK1
IRAK2
BTK
K63
TRAF6
TRAF3
TAB1/2/3
TAK1
TBK1
IKKε
K63
IKKαβγ
MAPK
IRF5
IRF7
Induced by IFN-β
K48
IkB
NF-kB
NF-kB
AP-1
IRF3
Nucleus
AP-1
NF-kB
TNF-α
pro-IL-1β
AP-1
IRF3
NF-kB
IFN-β
IRF7
IFN-α
b
ssRNA
CpG-DNA
Endolysosome
TLR7
TLR9
MyD88
IRAK4
IRAK1
IKKα
IRAK2
BTK
Opn
K63
TRAF6
TRAF3
TAB1/2/3
TAK1
IRF5
IRF7
Constitutively expressed
K63
IKKαβγ
MAPK
K48
IkB
NF-kB
NF-kB
AP-1
IRF7
IFN-α
AP-1
NF-kB
TNF-α
pro-IL-1β


RNA
RNA
DNA
LGP2
RIG-I
MDA5
cGAS
K63
TRIM25
+ATP
CARD
cGAMP
IPS-1
STING
STING
ER
ER
Mitochondrion
TRADD
RIP1
FADD
Casp8/10
TRAF6
K63
TRAF3
TANK
NAP1
SINTBAD
MEKK1
IKKαβγ
MAPK
TBK1
IKKε
K48
IkB
NF-kB
IRF7
Induced
by IFN-β
NF-kB
AP-1
IRF3
Nucleus
AP-1
NF-kB
TNF-α
pro-IL-1β
AP-1
IRF3
NF-kB
IFN-β
IRF7
IFN-α


Flagellin
Anthrax lethal toxin
MDP
PGN, MDP, lipoproteins,
DNA, RNA, uric acid crystals,
amyloid-β, asbestos, silica, alum
K+
ATP
?
Panx-1
P2X7
Bacterial
Type III / Type IV
secretion systems
Bacterial
pore-forming
toxins
low [K+]
high [ROS]
HSP90
SGT1
NLR
CARD
LRR
NACHT
IPAF
Pyrin
domain
FIIND
NLRP1
NLRP3
PYD
ATP
ADP
sensors
adaptors
pro-
caspases
Inflamma-
some
ASC
CARDINAL
Casp-1
Casp-5
Active
Caspases
Cytokine
Maturation
pro-IL-1β
pro-IL-18
IL-1β
IL-18
INFLAMMATION

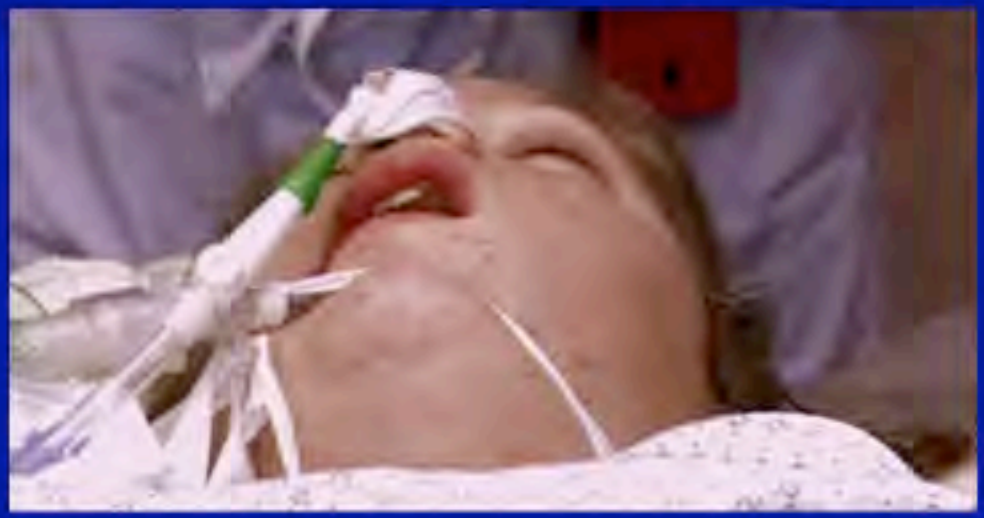


adapted from Teunis B, et al. Signalling through C-type lectin receptors, Nature Reviews Immun;July 2009, vol 9.

What does all this knowledge do for us?

Knowing how the immune system "sees" microbes during the very first seconds and minutes after infection, we can perhaps:

- Mitigate the intense inflammatory response that causes injury, while keeping the infection itself under control with antibiotics.



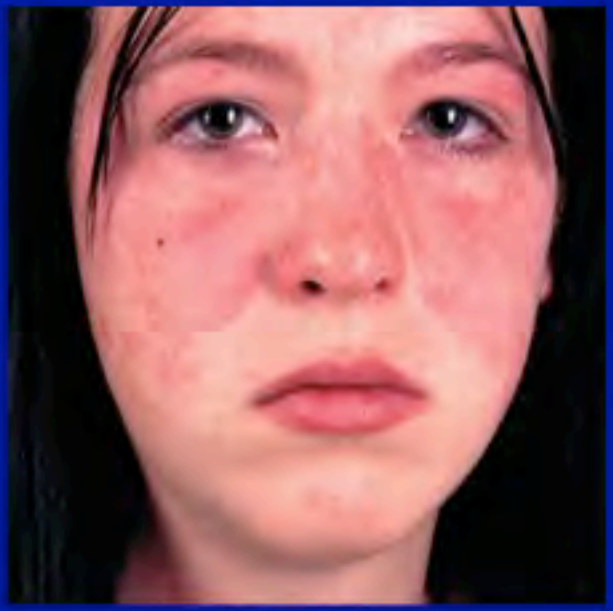
- Diagnose and treat certain immune deficiency diseases caused by failure of the sensing system.

- Design more effective vaccines, that have known molecular targets and lower toxicity than existing vaccines.

The legacy of our struggle against microbes is immunity.

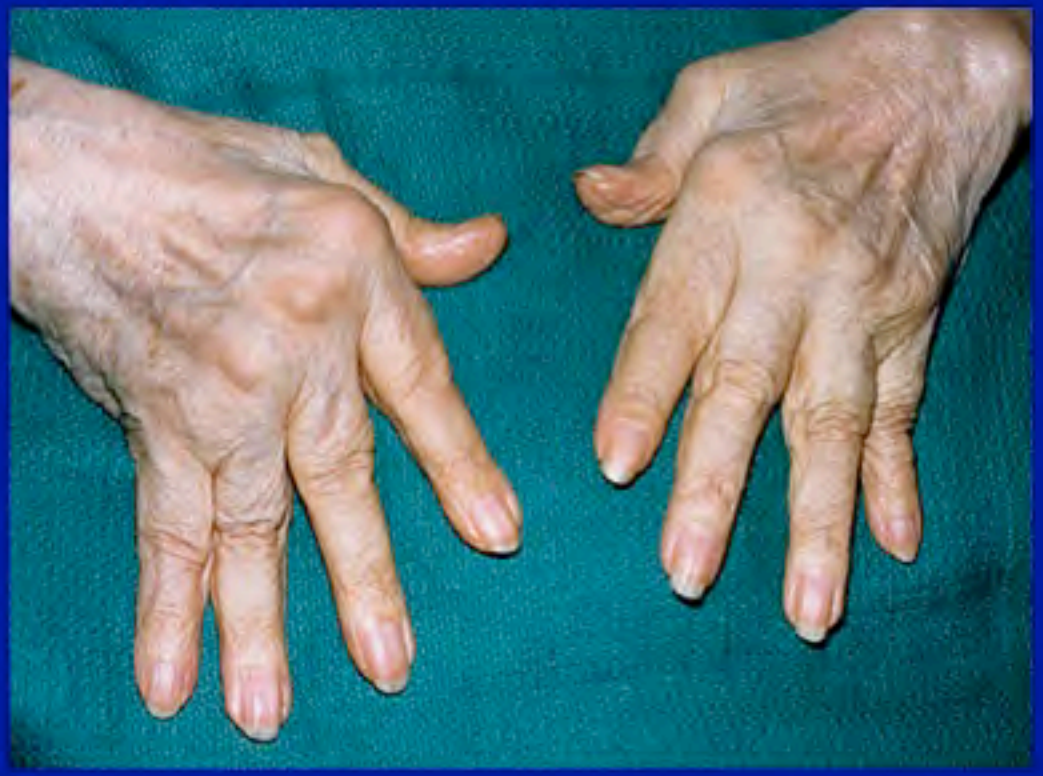
One legacy of immunity is autoimmunity.

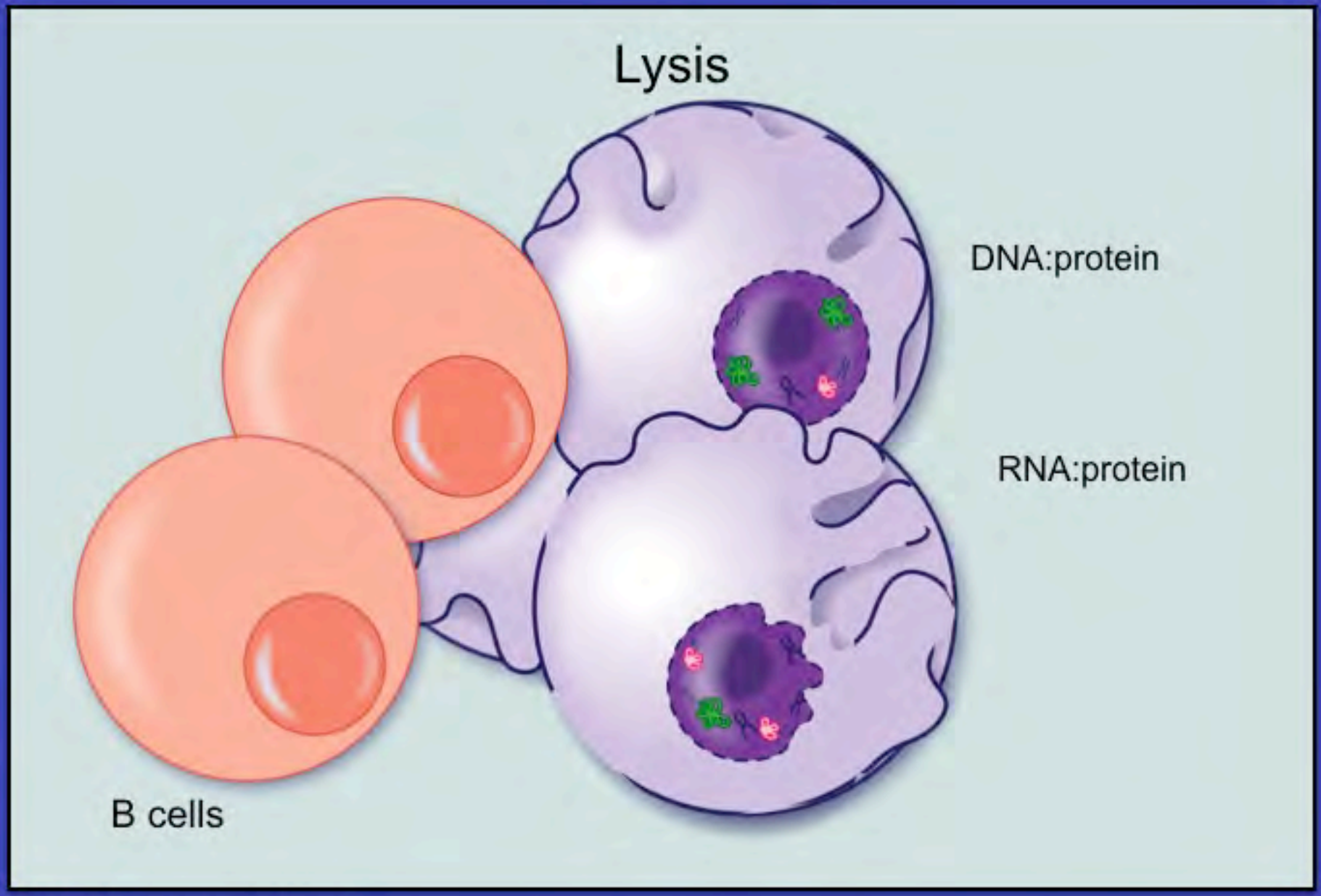
- Treat certain inflammatory and autoimmune diseases much more specifically than in the past. Systemic lupus erythematosus (SLE) is a particularly promising target.



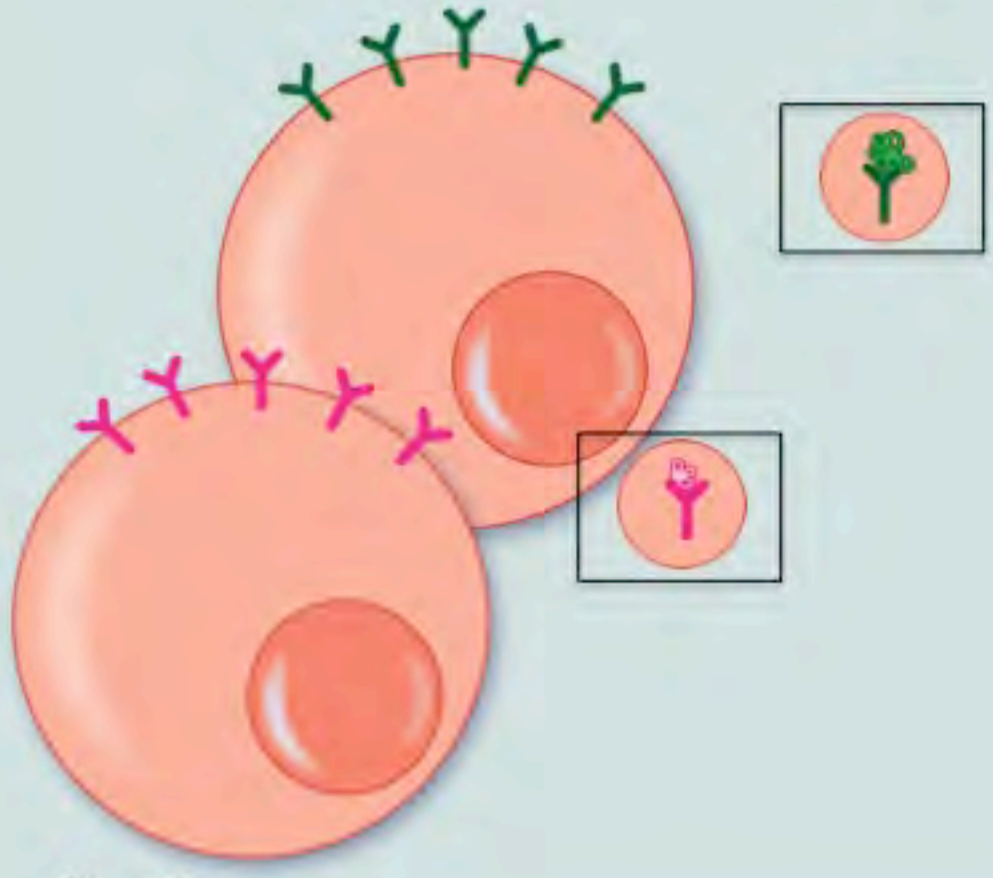
SLE

Rheumatoid arthritis





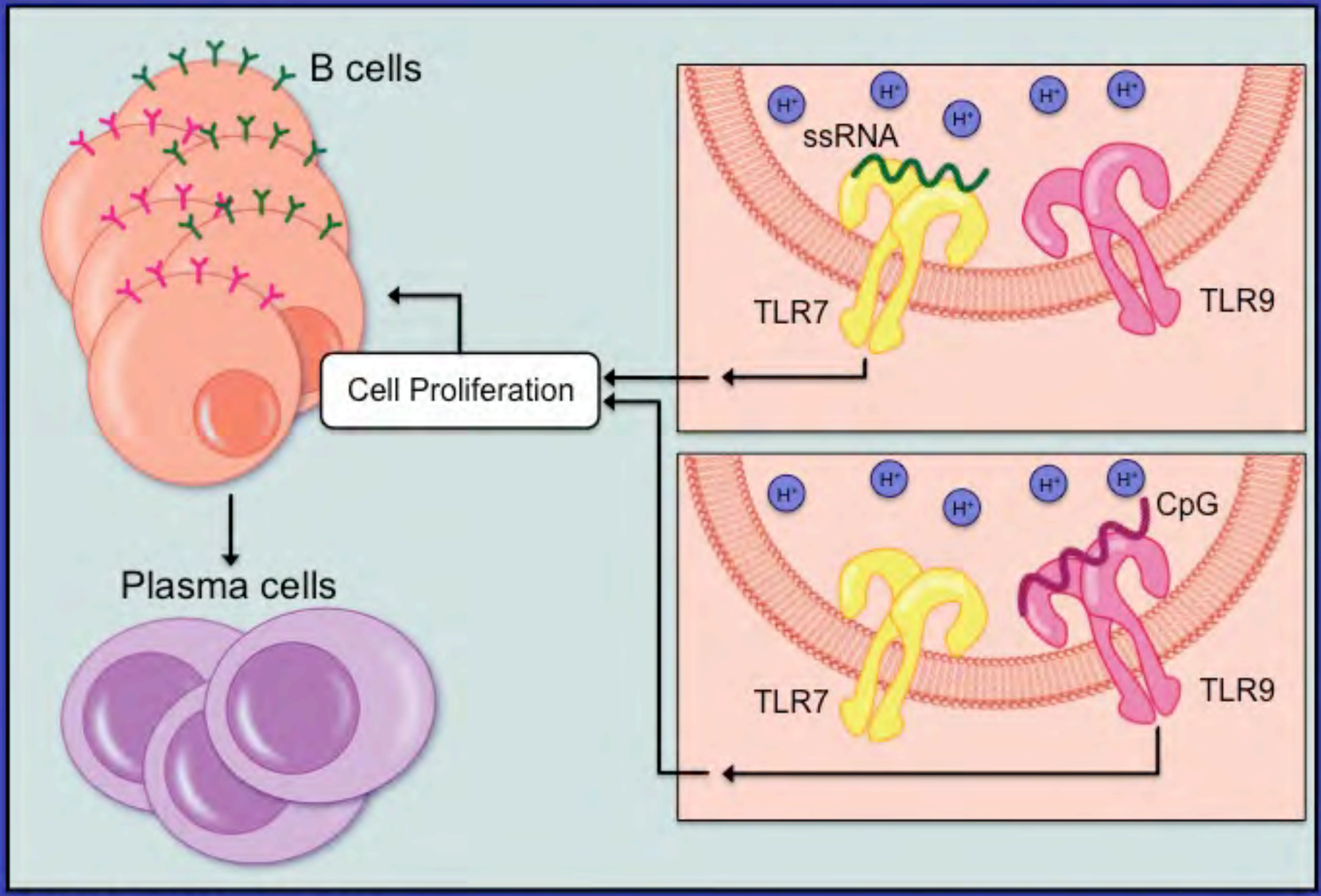
Leadbetter, EA, et al. Nature. 2002 Apr 11;416(6881):603-7.



DNA:protein

RNA:protein

Leadbetter, EA, et al. Nature. 2002 Apr 11;416(6881):603-7.



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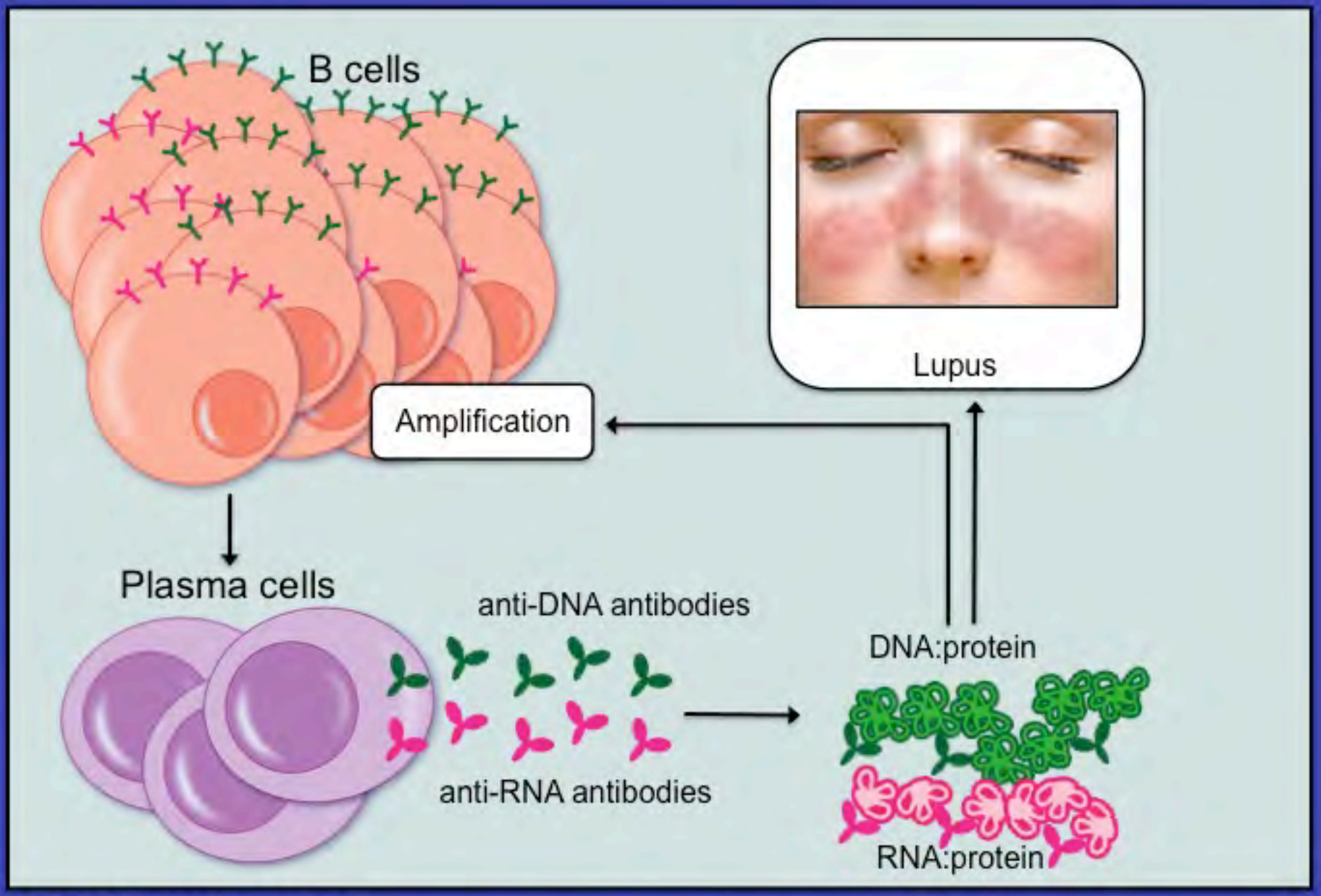

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August 5, 2014