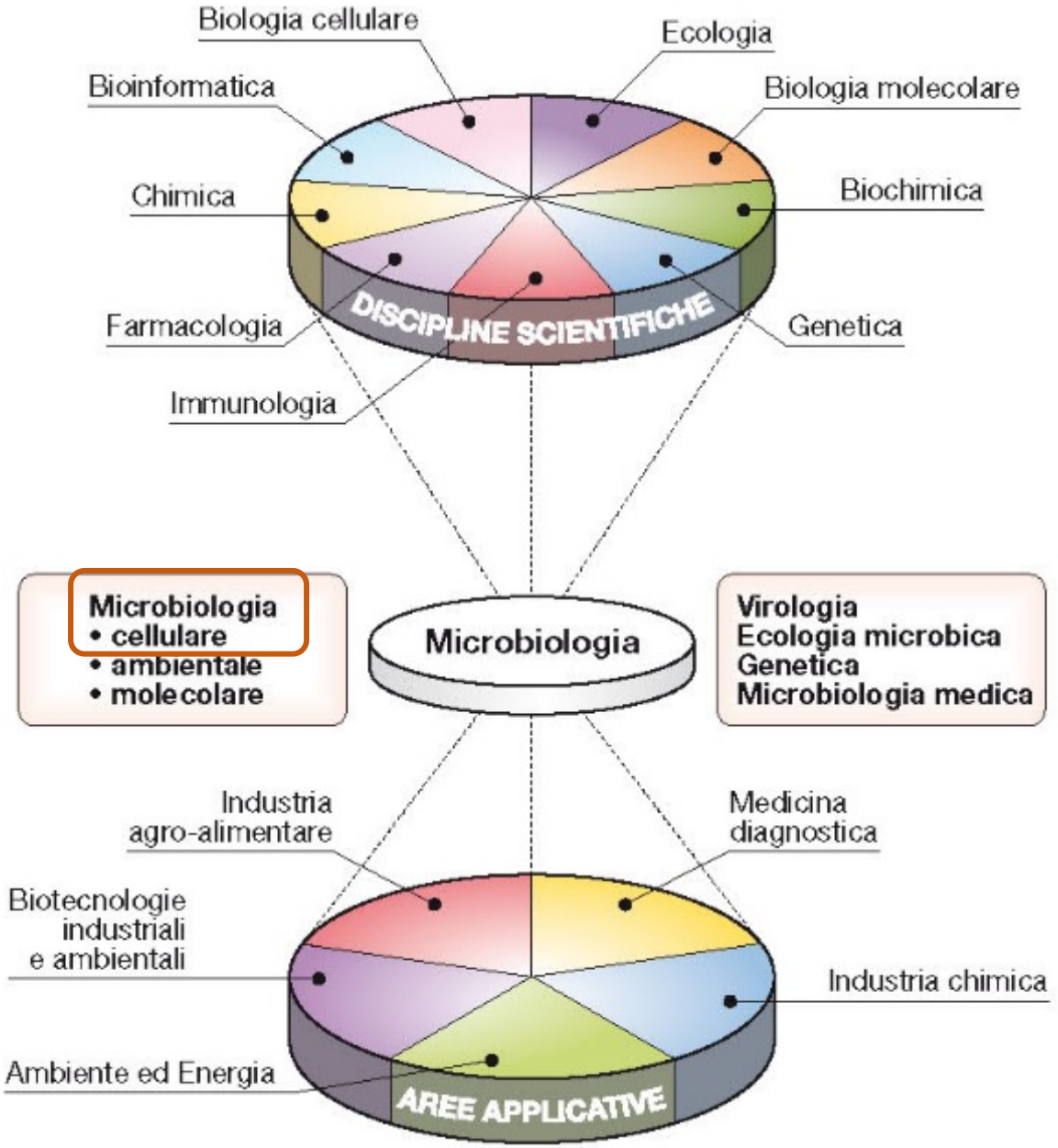
Microbiologia cellulare e vaccinologia 2023/2024


cellular
microbiology
virulence
microscopy
uptake
bacteria
modify
vesicle
coined
trafficking
mutual
research
dependency
eukaryotic
microbe
promote
microbial
discipline
internalize
projections
cell
inhibit
investigation
synthesis
bridges
fluorescence
transduction
formation
polymerization
cellular
tools
degradation
processes
phagocytes
toxin
pathogenic
emergence
purified
conferences
molecular
engulf
bacterial
elicit
biology
clarified
regulation
expanded
influence
atomic
microbiology
counterparts

I molteplici aspetti della moderna microbiologia



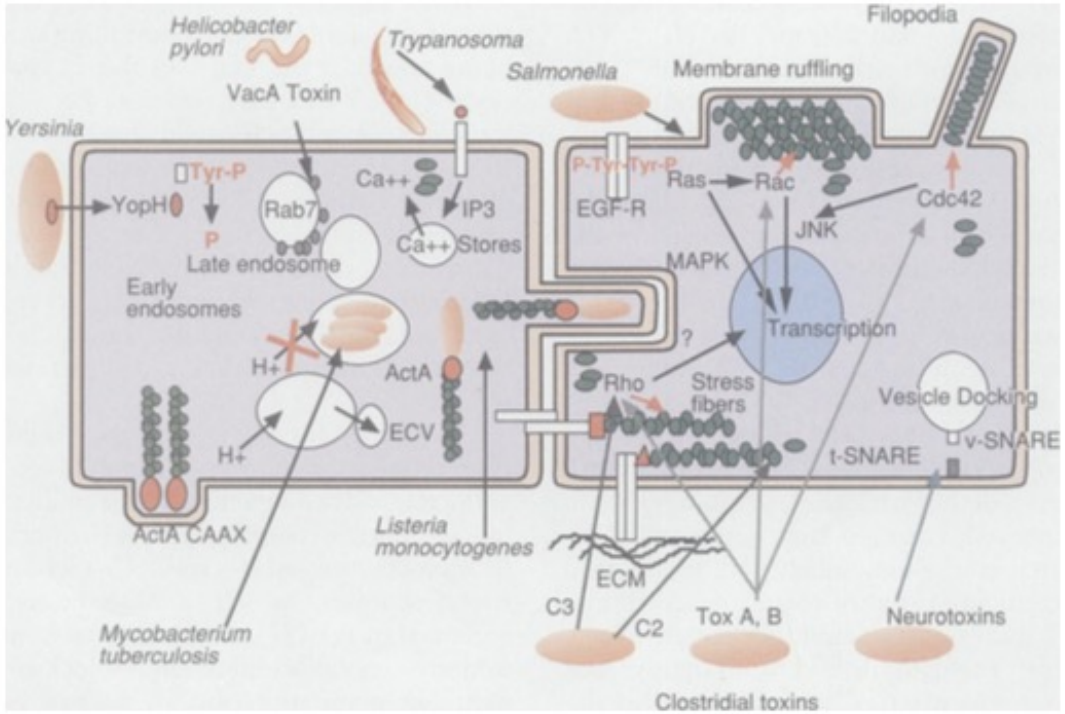
Cellular Microbiology Emerging

P. Cossart, P. Boquet, S. Normark, R. Rappuoli

A new discipline, cellular microbiology, is emerging at the interface between cell biology and microbiology. Traditional cell biological approaches are already widely used to unravel the tactics microbes utilize to infect their hosts, but the use of pathogens to tackle questions in cell biology is just now yielding promising approaches and elegant results. Two meetings, in 1989 and 1991 (*1*), laid the groundwork for the field, and a third meeting in 1995 highlighted recent progress (*2*).

A major focus of this new field is the actin network, which together with intermediate filaments and microtubules constitute the cytoskeleton. The rapid assembly and disassembly of actin microfilaments is essential for phagocytosis, motility, cell division, and adhesion to a substratum or to another cell. Yet, the signaling pathways that control actin dynamics are poorly understood. Bacteria that can be genetically manipulated and parasites can provide tools to dissect these control pathways. When certain bacteria, such as *Salmonella* and *Shigella*, infect cells, they mimic the action of epidermal growth factor (EGF), inducing membrane ruffling and active actin polymerization (*3*–*5*) (see figure). The ruffling leads to internalization of the bacteria.

The internalization of other pathogens occurs without membrane ruffling or even actin polymerization. The parasite *Trypanosoma cruzi* enters cells by triggering a combination of events—a transient increase in cytosolic free calcium, rapid rearrangement of the cortical actin cytoskeleton, and lysosome recruitment and clustering at the invasion site (*6*, *7*). Lysosomes contribute membrane for the formation of the parasitophorous vacuole. Disruption of cortical actin by the increase in local calcium allows lysosomes to migrate and fuse, a phenomenon also regulated by calcium. Phospholipase C



Pathogenic bacteria interfere with numerous eukaryotic cell functions, providing a sophisticated tool kit for cell biologists.

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Microreview

Luglio 1999

Cellular Microbiology is launched

Stanley Falkow
Department of Microbiology and Immunology, Stanford University School of Medicine, Fairchild Building, D039, 299 Campus Drive, Stanford, CA 94305-5124, USA.

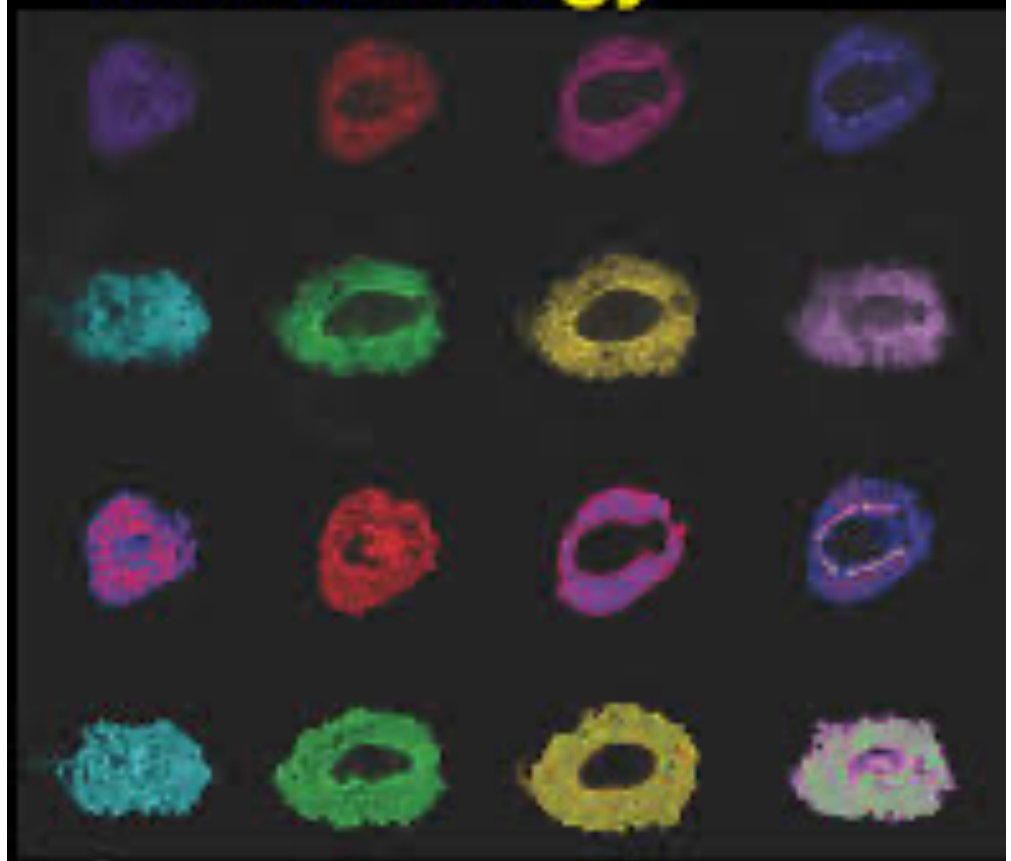
I was given the privilege of writing the first article for the journal *Cellular Microbiology*. In doing so, perhaps I can be forgiven if I indulge myself in some personal reflections. Until the 1980s, my professional life had been spent in the earnest pursuit of understanding medically important bacteria. Nevertheless, like most other ageing scientists, I am the 'prisoner' of the students with whom I work. I depend on them to be smarter and to learn new things better and faster than I do. Thus, when Ralph Isberg described the *Yersinia* protein invasin (Isberg and Falkow, 1985), I suddenly was plunged from the world of bacterial adhesins and toxins to the study of how bacteria actually invade host cells. I began to try to understand the cell biology of eukaryotes – those creatures I had considered in the

Of course, we were not the first microbiologists to see it was time to look seriously at the cell biology of microbe–host interactions, nor were cell biologists ignorant of the advantages of using microbial virulence factors as probes to dissect the biology of animal cells. However, the title and subject matter of this journal is 'cellular microbiology', a term that was coined in 1996 by P. Cossart, P. Boquet, S. Normark and R. Rappuoli to describe more precisely an emerging scientific discipline bridging cell biology and microbiology (Cossart *et al.*, 1996). The idea first began to blossom at a scientific meeting held in Arolla, Switzerland, in the summer of 1991, which brought together scientists who were exploring cell biological tools to answer questions of bacterial pathogenesis and cell biologists who had discovered that the fundamental questions of cell biology could be productively addressed by using microbial pathogens and bacterial toxins as cellular probes.

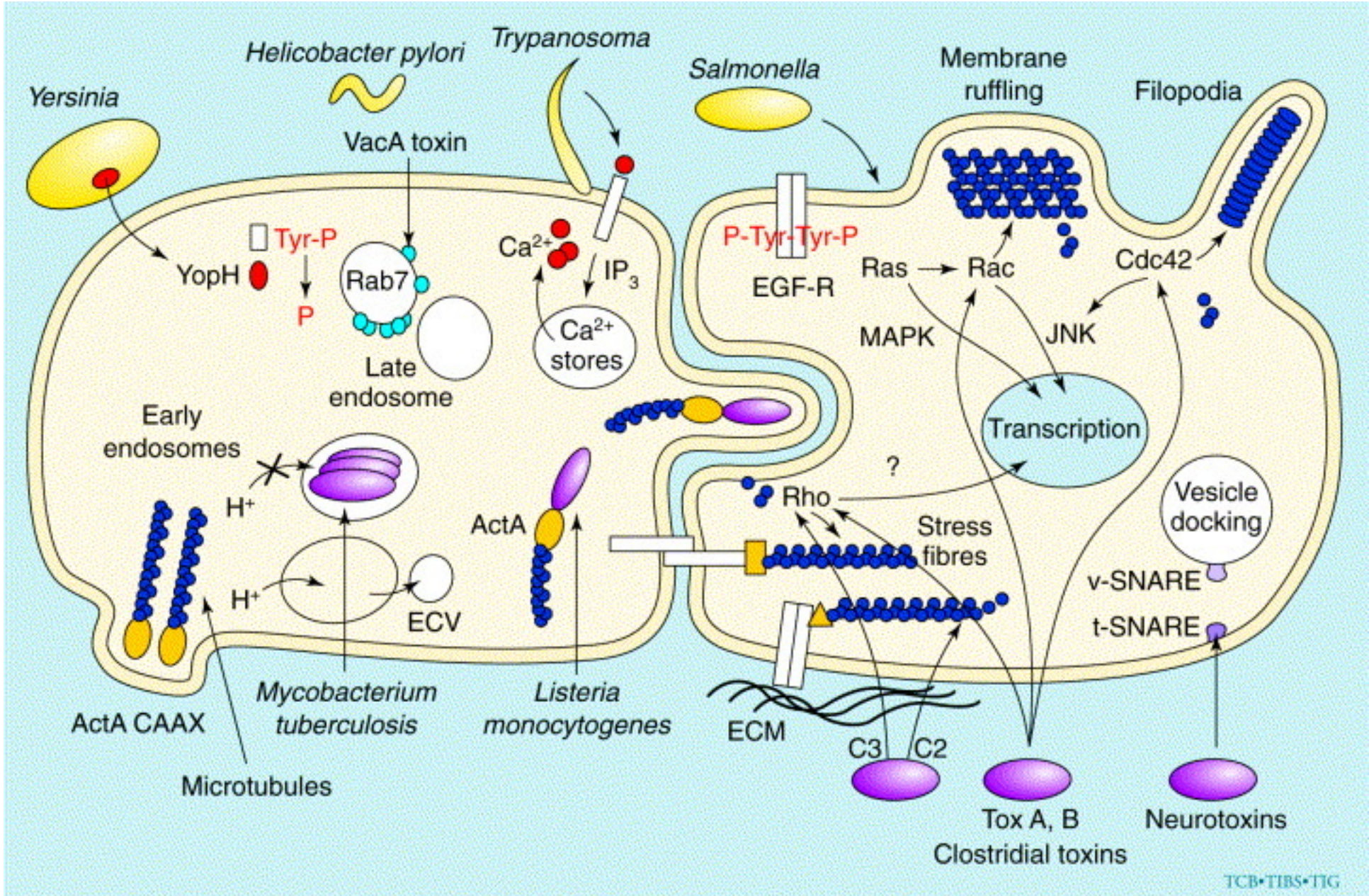
Purified bacterial toxins and viruses had been used for decades by cell biologists. It was not necessary to know very much in the way of microbiology to use them as biochemical probes of cell biological function. In particular,

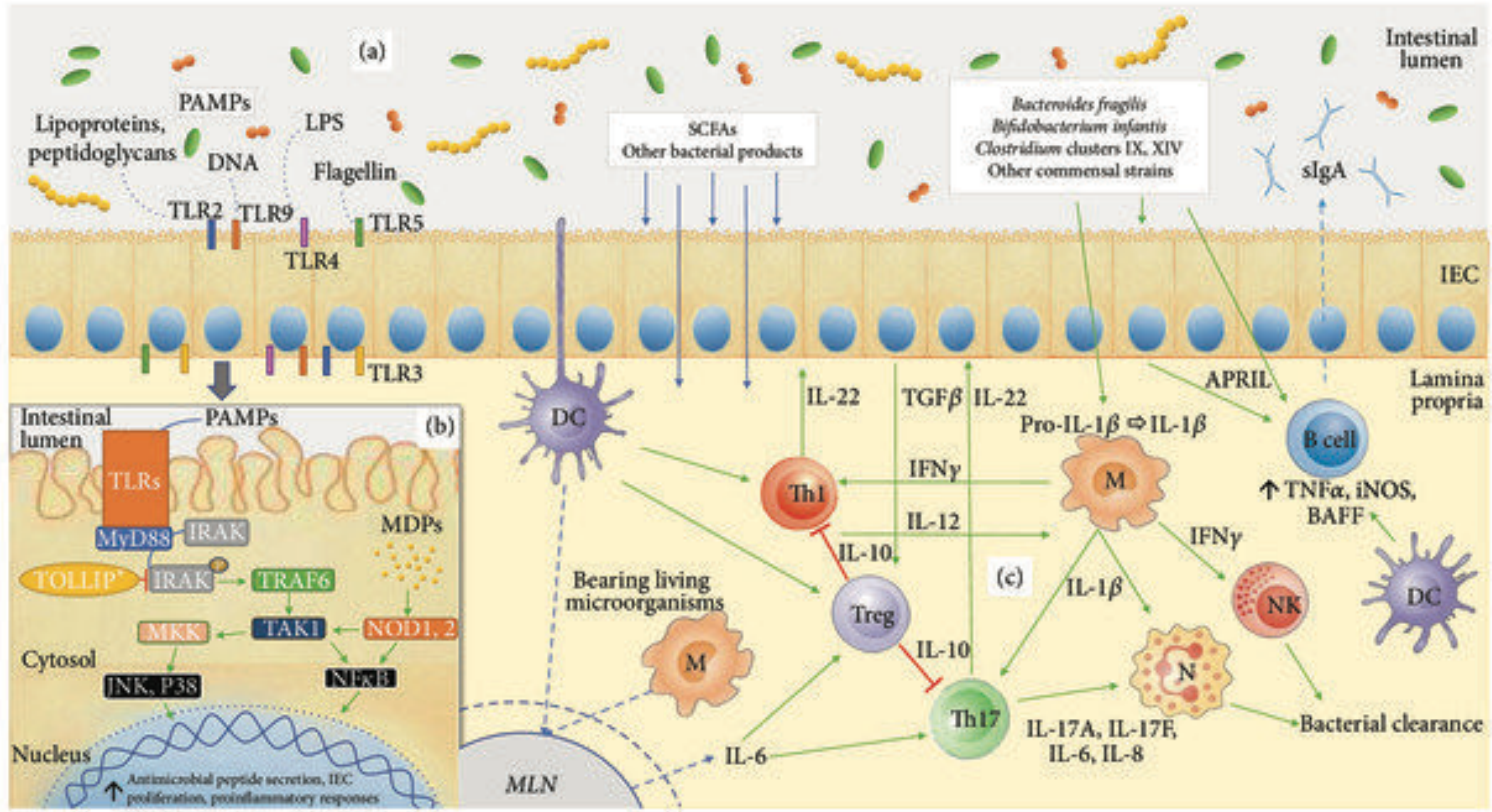
VOLUME 13 NUMBER 2 FEBRUARY 2011

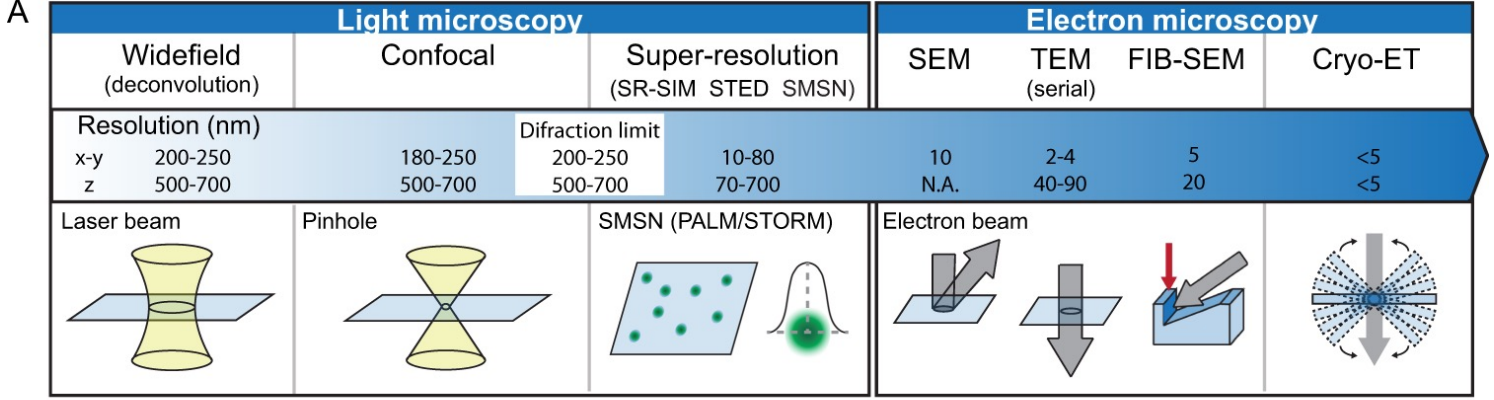
cellular microbiology

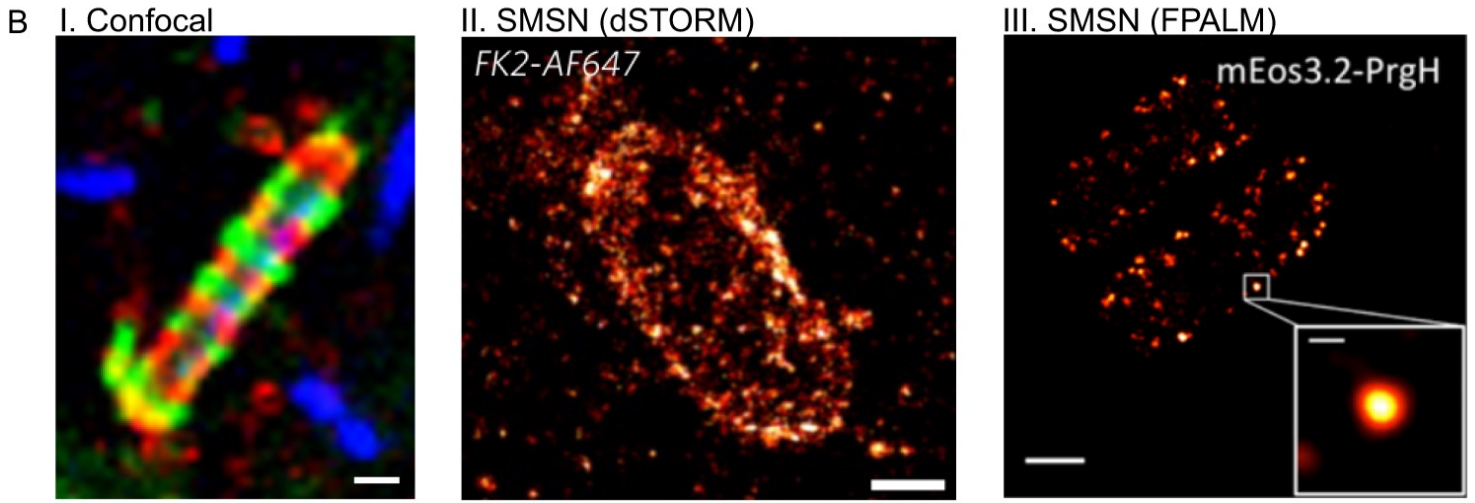


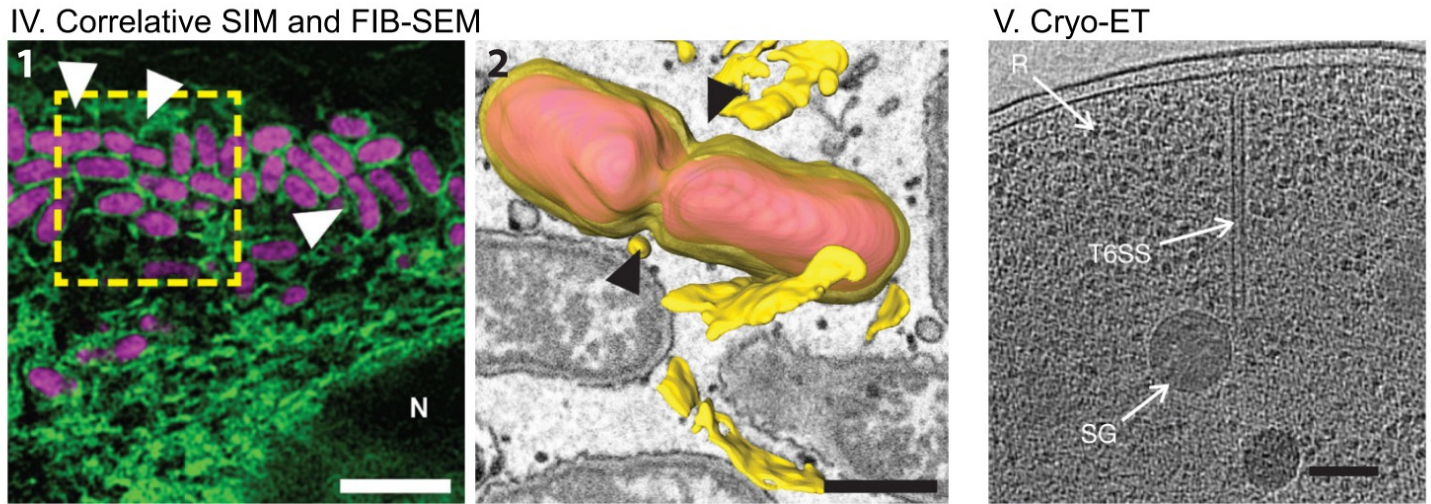
WILEY-BLACKWELL


Yersinia
Helicobacter pylori
Trypanosoma
Salmonella
Membrane ruffling
Filopodia
VacA toxin
Tyr-P
YopH
P
Rab7
Late endosome
Ca2+
IP3
Ca2+ stores
P-Tyr-Tyr-P
EGF-R
Ras
Rac
Cdc42
MAPK
JNK
Transcription
Early endosomes
H+
H+
ECV
ActA
?
Rho
Stress fibres
Vesicle docking
v-SNARE
t-SNARE
ActA CAAX
Mycobacterium tuberculosis
Listeria monocytogenes
ECM
C3
C2
Microtubules
Tox A, B
Clostridial toxins
Neurotoxins
TCB•TIBS•TIG


(a)
Intestinal lumen
PAMPs
Lipoproteins, peptidoglycans
DNA
LPS
Flagellin
TLR2
TLR9
TLR4
TLR5
TLR3
SCFAs
Other bacterial products
Bacteroides fragilis
Bifidobacterium infantis
Clostridium clusters IX, XIV
Other commensal strains
sIgA
IEC
Lamina propria
(b)
Intestinal lumen
PAMPs
TLRs
MyD88
IRAK
MDPs
TOLLIP
IRAK
TRAF6
MKK
TAK1
NOD1, 2
Cytosol
JNK, P38
NFκB
Nucleus
↑ Antimicrobial peptide secretion, IEC proliferation, proinflammatory responses
DC
IL-22
TGFβ
IL-22
Pro-IL-1β ⇨ IL-1β
APRIL
B cell
↑ TNFα, iNOS, BAFF
Th1
IFNγ
M
IL-12
IL-10
IFNγ
Bearing living microorganisms
M
Treg
(c)
IL-1β
NK
DC
IL-10
N
Th17
IL-17A, IL-17F, IL-6, IL-8
Bacterial clearance
IL-6
MLN


A
Light microscopy
Widefield
(deconvolution)
Confocal
Super-resolution
(SR-SIM STED SMSN)
Electron microscopy
SEM
TEM
(serial)
FIB-SEM
Cryo-ET
Resolution (nm)
Difraction limit
x-y 200-250 180-250 200-250 10-80 10 2-4 5 <5
z 500-700 500-700 500-700 70-700 N.A. 40-90 20 <5
Laser beam
Pinhole
SMSN (PALM/STORM)
Electron beam


B
I. Confocal
II. SMSN (dSTORM)
FK2-AF647
III. SMSN (FPALM)
mEos3.2-PrgH


IV. Correlative SIM and FIB-SEM
1
N
2
V. Cryo-ET
R
T6SS
SG

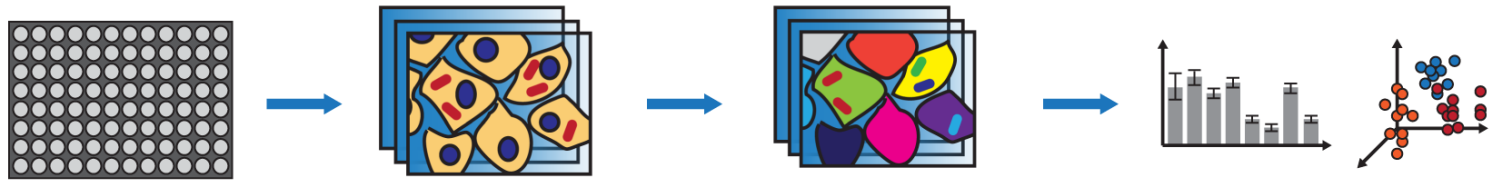
High-throughput / high-content imaging screenings

Library to be screened:
- siRNA
- CRISPR-Cas9
- Transposon
- Chemical

Acquisition
- Automated microscope
- Liquid handling robot
- Coupled incubator

Analysis
- Image processing
- Object identification
- Feature extraction
- Machine learning segmentation and classification

Quantification
- Multiparametric analysis
- Principal component analysis
- Hit detection and validation

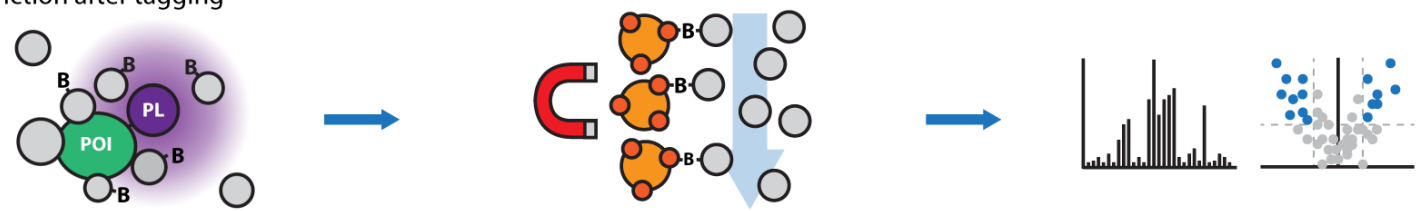


Biochemical mapping

Tagging of protein of interest (POI)
- Different types of proximity labelling (PL) enzymes available
- Verification of protein localisation and function after tagging

Purification of biotinylated proteins
- Pull down of biotinylated proteins with streptavidin coated beads

Identification of proteins
- Mass spectrometry
- Quantitative proteomics
- Hit detection and validation

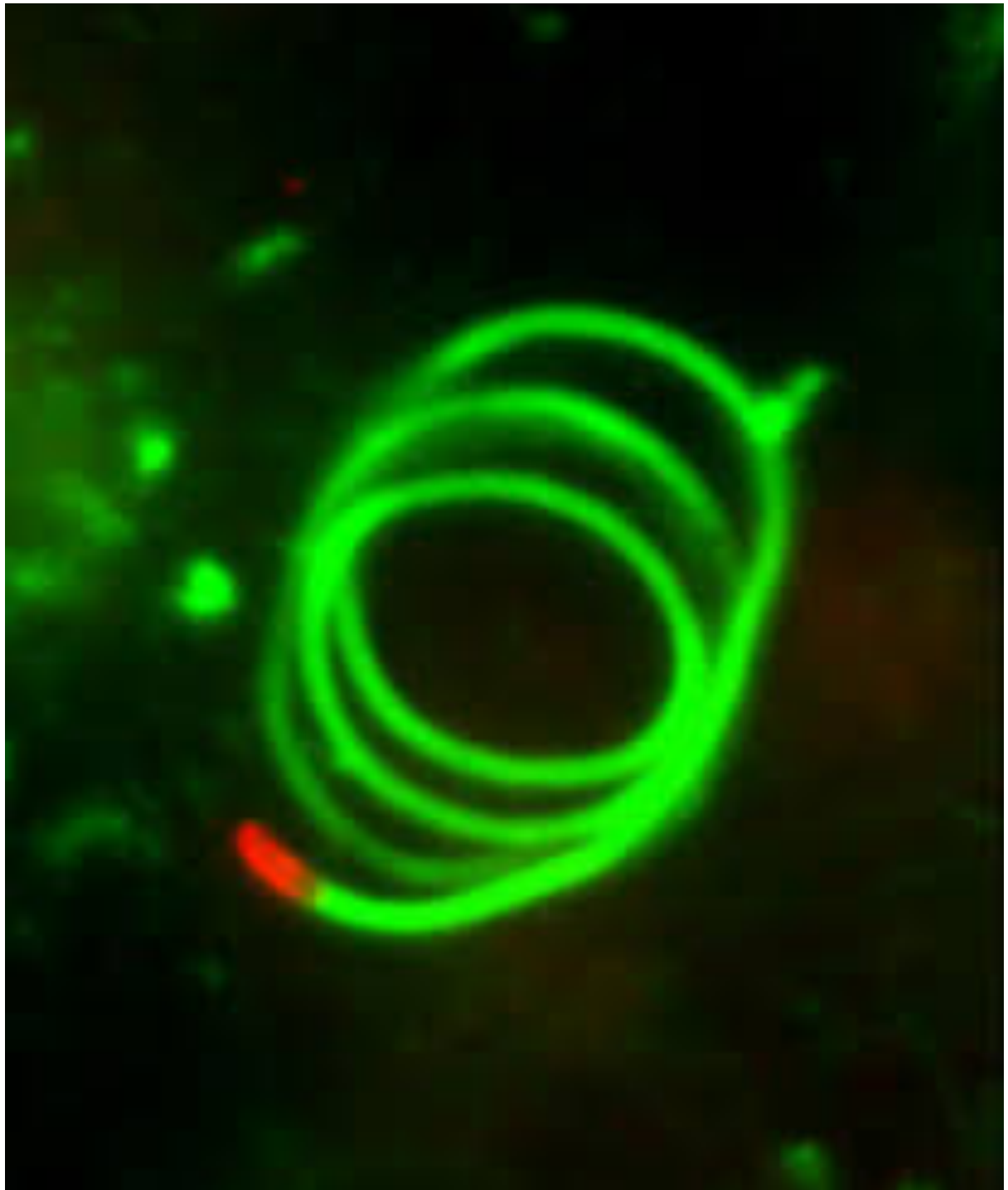


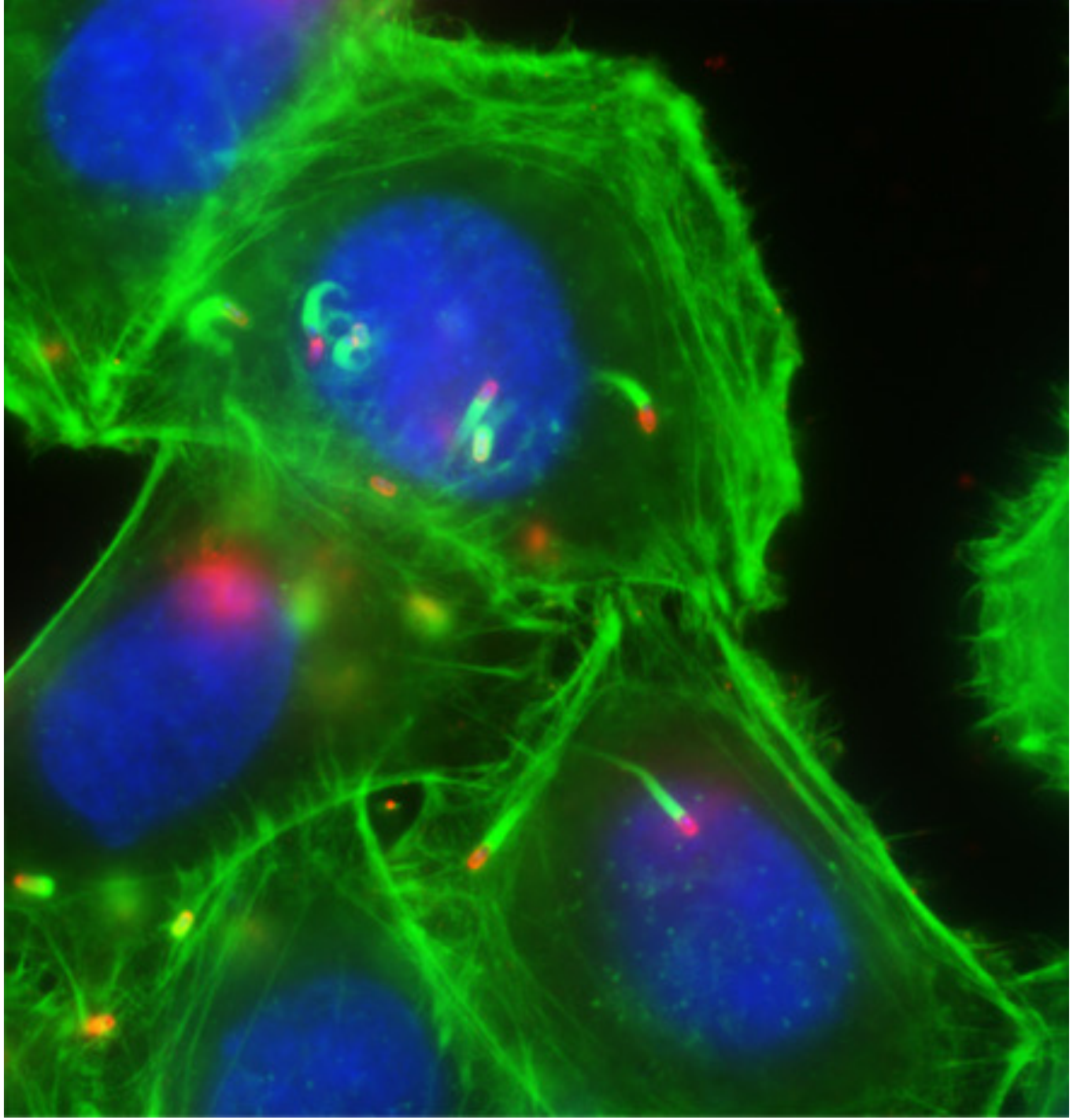
Dual RNA-sequencing

RNA isolation from infected sample
- Types of RNA: coding and non-coding sequences
- Depletion of abundant and non informative rRNA

Identification of transcripts
- Reverse transcription
- Deep sequencing
- Mapping of reads

Transcript analysis
- Differential expression profiles
- Pathway enrichment analysis
- Identification of non-coding regulatory elements





I NOBEL CHE HANNO CONTRIBUITO ALLO SVULUPPO E ALL'EVOLUZIONE DELLA "CELLULAR MICROBIOLOGY"

Warren & Marshall receiving the Nobel Prize from the King Carl XVI Gustaf of Sweden


Robin Warren


Barry Marshall

Stockholm Concert Hall, 10 December 2005

Photo: Mosimann for Balzan

Bruce A. Beutler

Photo: Mosimann for Balzan

Jules A. Hoffmann

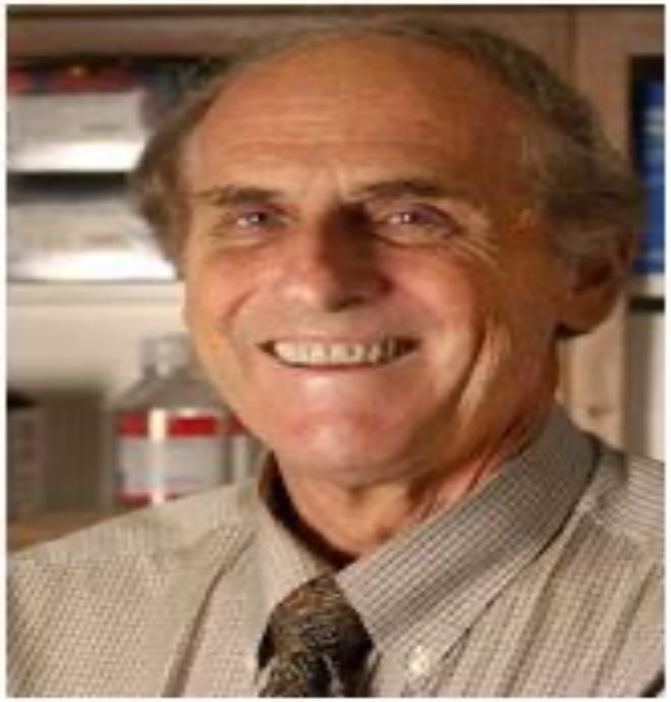
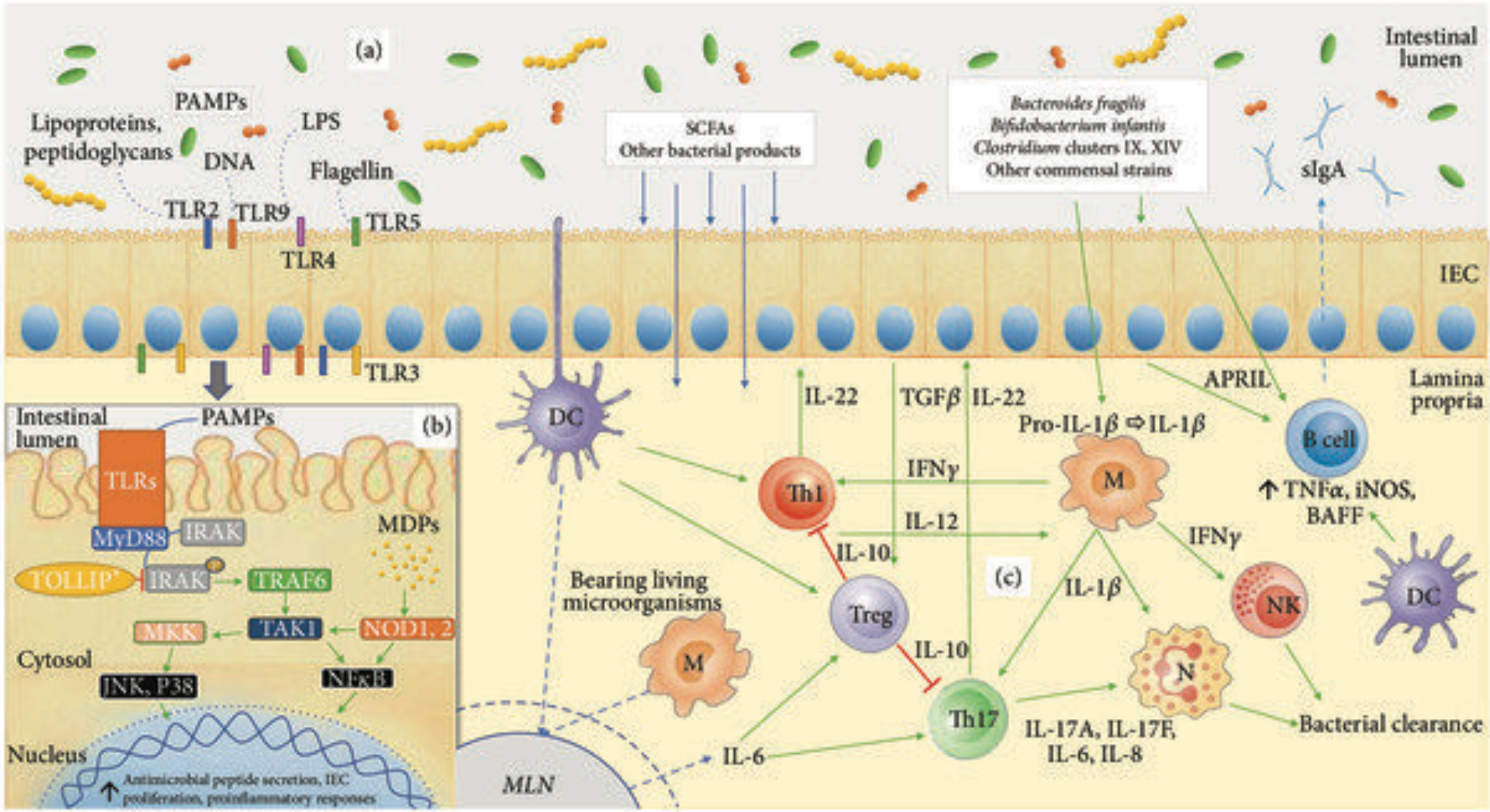


Photo: Rockefeller University Press

Ralph M. Steinman

The Nobel Prize in Physiology or Medicine 2011 was divided, one half jointly to Bruce A. Beutler and Jules A. Hoffmann *"for their discoveries concerning the activation of innate immunity"* and the other half to Ralph M. Steinman *"for his discovery of the dendritic cell and its role in adaptive immunity"*.

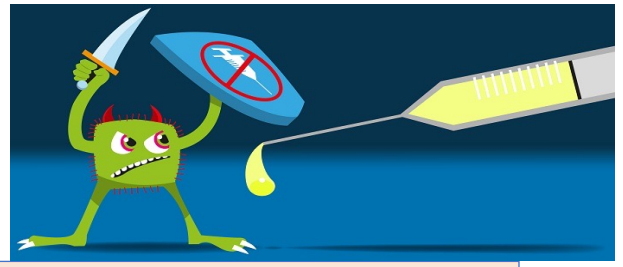

(a)
PAMPs
Lipoproteins, peptidoglycans
DNA
LPS
Flagellin
TLR2
TLR9
TLR4
TLR5
TLR3
SCFAs
Other bacterial products
Bacteroides fragilis
Bifidobacterium infantis
Clostridium clusters IX, XIV
Other commensal strains
sIgA
Intestinal lumen
IEC
Lamina propria
(b)
Intestinal lumen
PAMPs
TLRs
MyD88
IRAK
MDPs
TOLLIP
IRAK
TRAF6
MKK
TAK1
NOD1, 2
Cytosol
JNK, P38
NFκB
Nucleus
↑ Antimicrobial peptide secretion, IEC proliferation, proinflammatory responses
DC
IL-22
TGFβ
IL-22
APRIL
Pro-IL-1β ⇨ IL-1β
B cell
↑ TNFα, iNOS, BAFF
Th1
IFNγ
M
IL-12
IL-10
IFNγ
Bearing living microorganisms
M
Treg
(c)
IL-1β
NK
DC
IL-10
N
Th17
IL-17A, IL-17F, IL-6, IL-8
Bacterial clearance
IL-6
MLN


Vaccinology


VACCINOLOGY

Cos'è un vaccino?

Cos’è un vaccino?



Un prodotto, usualmente composto da microrganismi uccisi o viventi e resi innocui o molecole di questi, che stimola il sistema immunitario a conferire immunità nei confronti di una specifica malattia indotta da tali microrganismi, proteggendo così gli individui dall’insorgenza della malattia.

L'immunità è lo stato di resistenza specifica, congenita o indotta, di un organismo a malattie infettive o a sostanze tossiche, per la formazione di anticorpi umorali o per lo sviluppo di immunità cellulare.

IMMUNITÀ

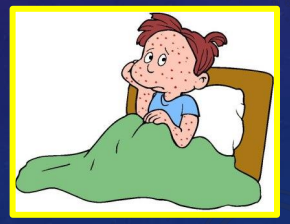
Resistenza dell'organismo, congenita o acquisita, all'azione di determinati germi patogeni o tossine.

IMMUNITÀ

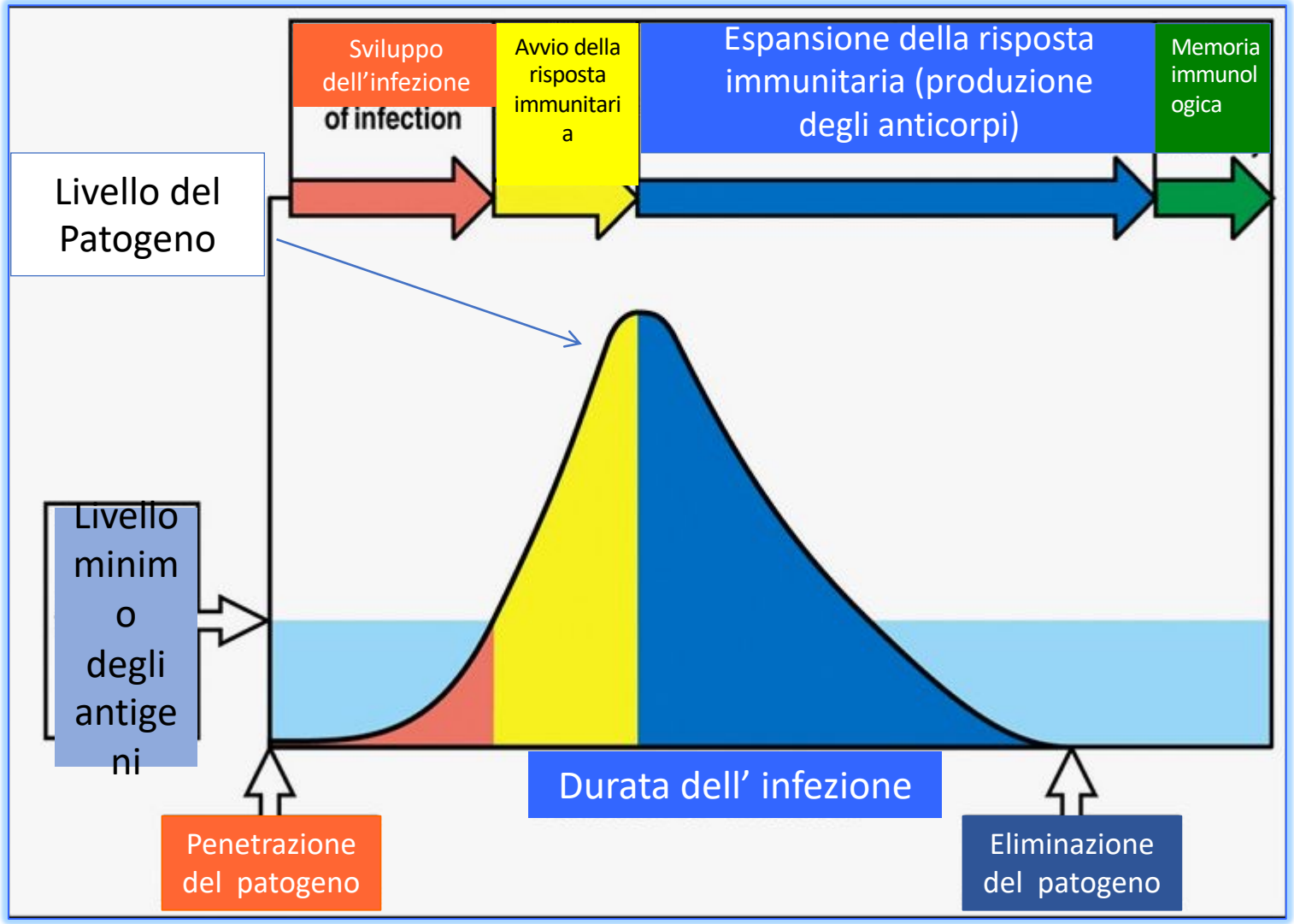
PASSIVA
Temporanea

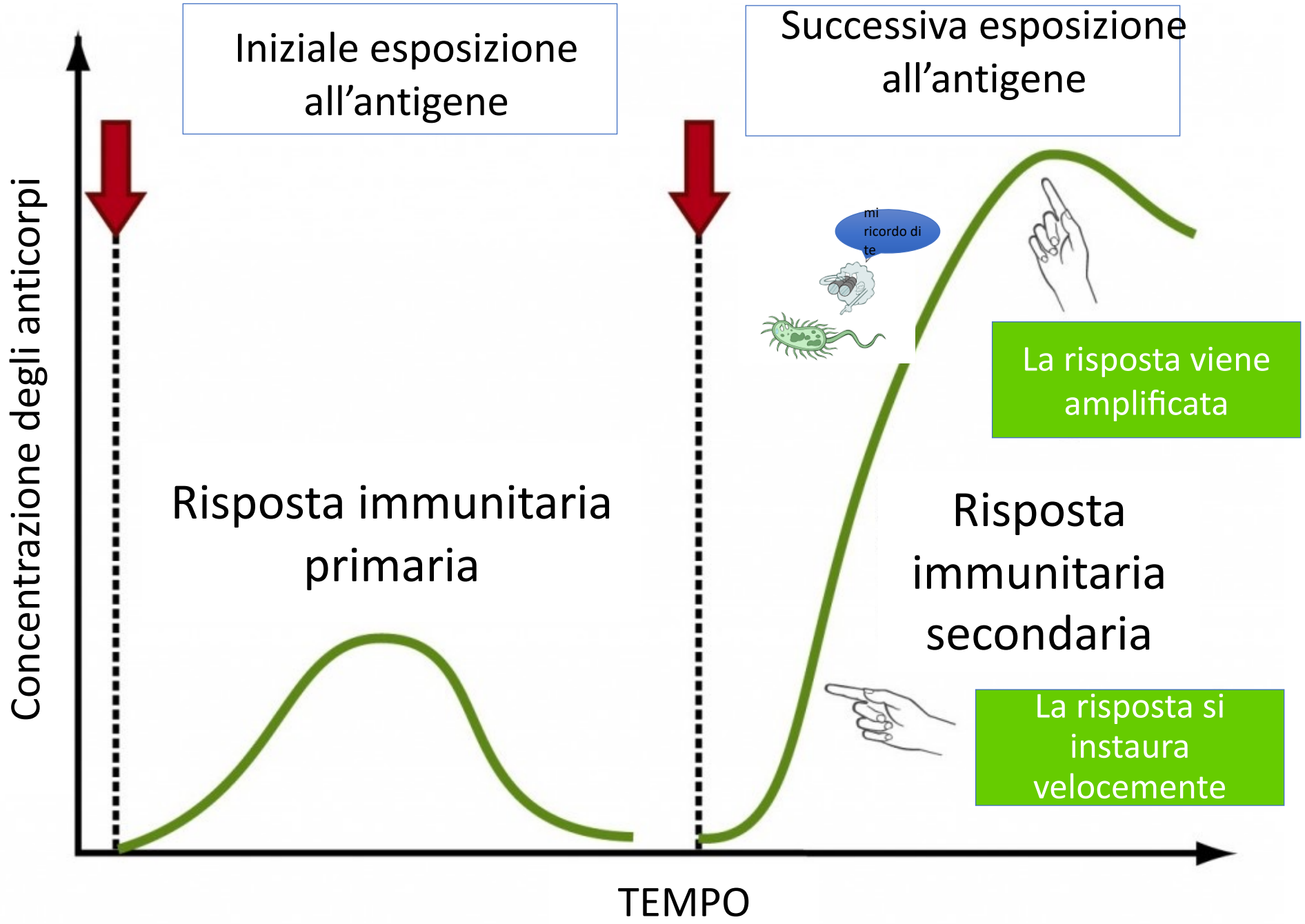
ATTIVA
Persistente o Permanente

- Anticorpi materni alla nascita
- Sieroprofilassi
- Infezioni o Malattie
- Vaccinazioni



Cosa avviene durante un'infezione….




Iniziale esposizione all'antigene
Successiva esposizione all'antigene
Concentrazione degli anticorpi
mi ricordo di te
La risposta viene amplificata
Risposta immunitaria primaria
Risposta immunitaria secondaria
La risposta si instaura velocemente
TEMPO

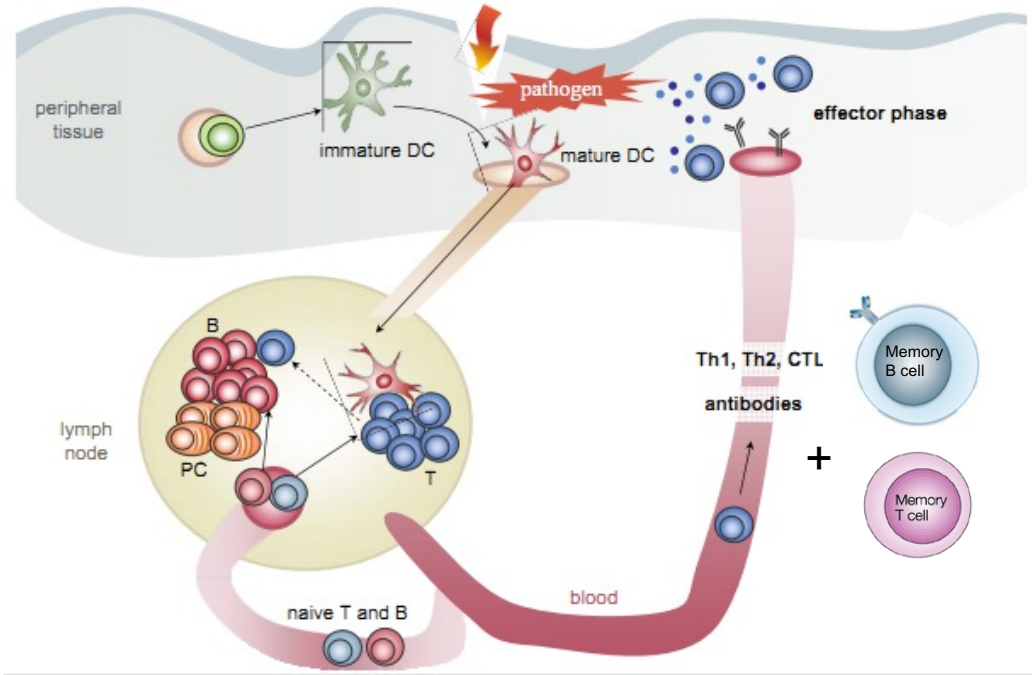
Generalità

- Farmaco profilattico allo scopo di prevenire una malattia infettiva
- Per una popolazione ampia
- Somministrato a persone sane

- Uno dei settori più innovativi delle ricerca medica
  - Risultati da dati empirici per molto tempo
  - Oggi controllati
  - Comprensione dei meccanismi coinvolti

Scopo di un vaccino

Farmaco <u>profilattico</u> allo scopo di prevenire una malattia infettiva



<u>chema generale della risposta nmunitaria primaria</u>

“Preparazione/Educazione” del sistema immunitario

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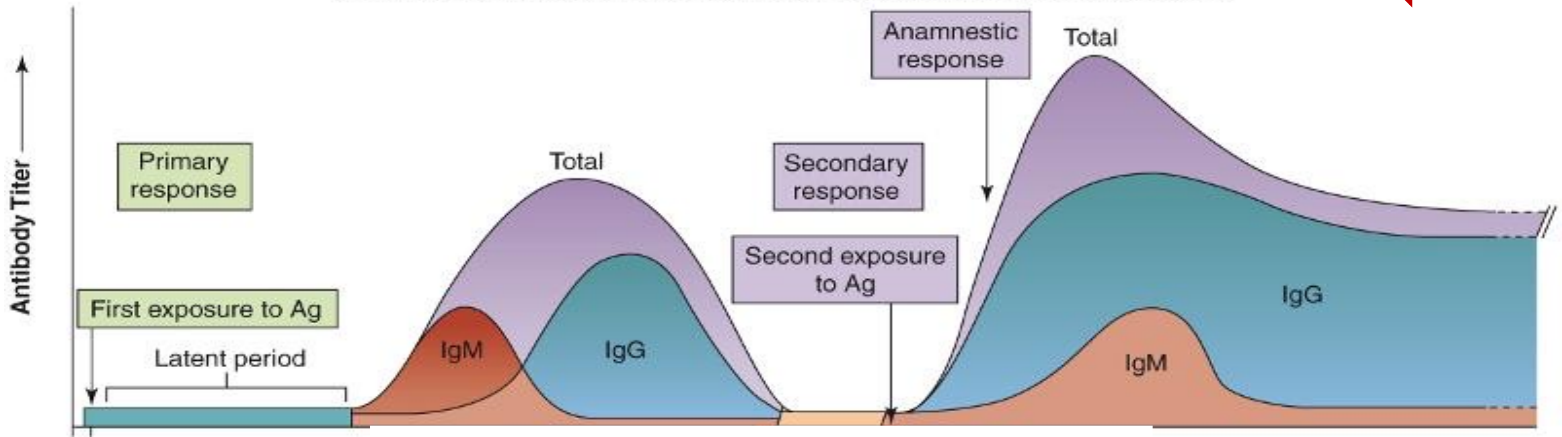
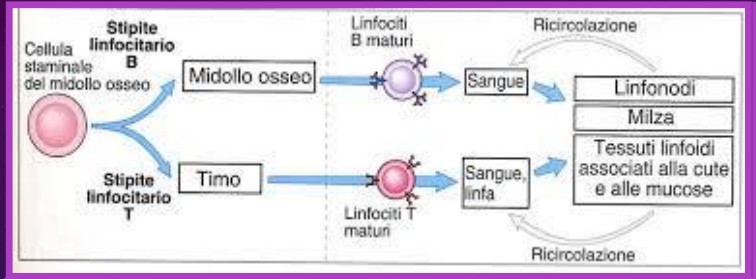


SISTEMA IMMUNITARIO

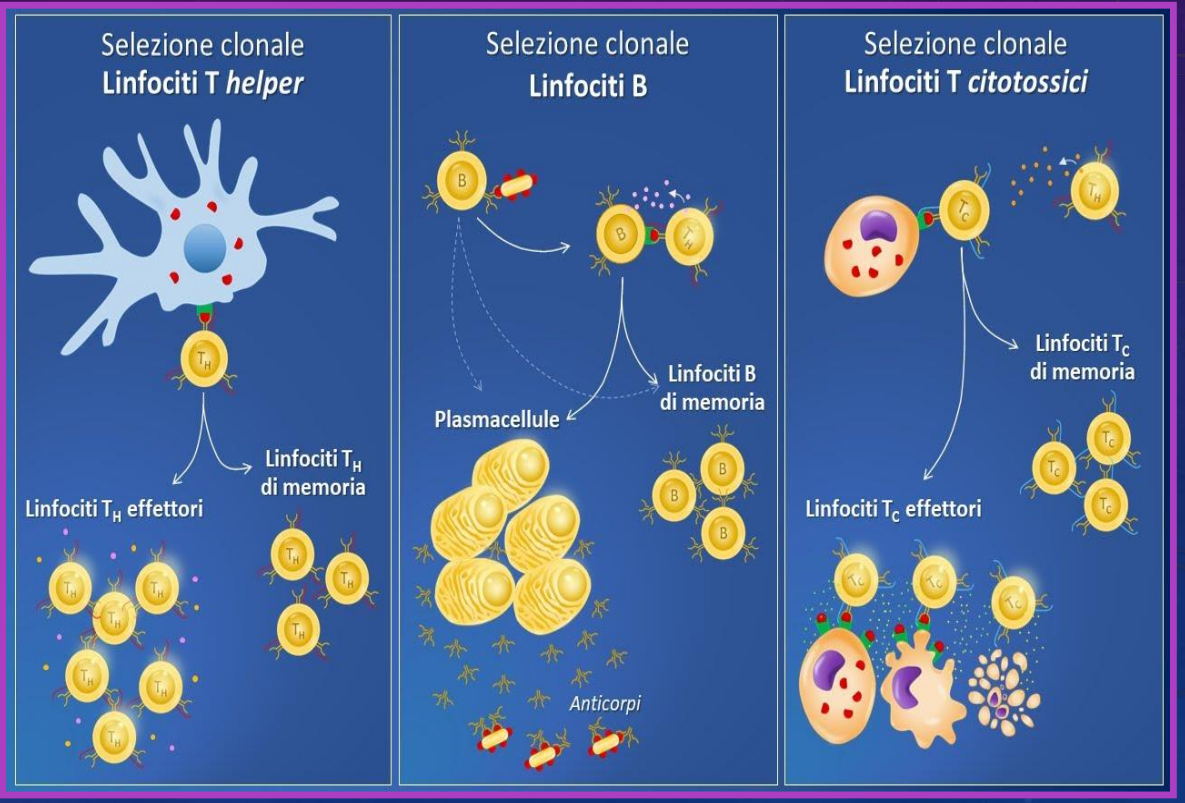


Linfociti T

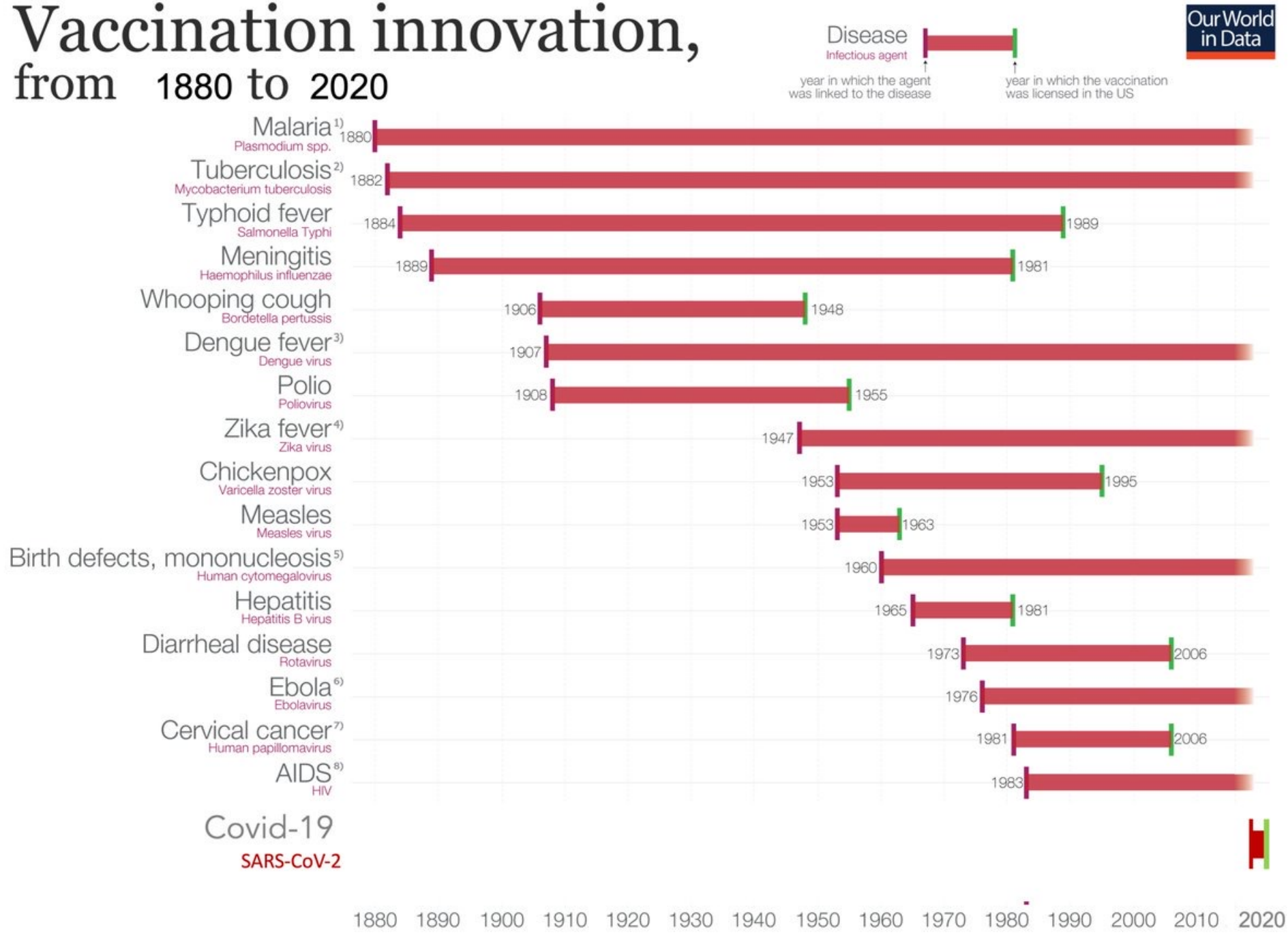
Nel timo imparano a riconoscere le cellule dell'organismo "cellule self", in circolo identificano gli antigeni estranei, "non self"

Linfociti B

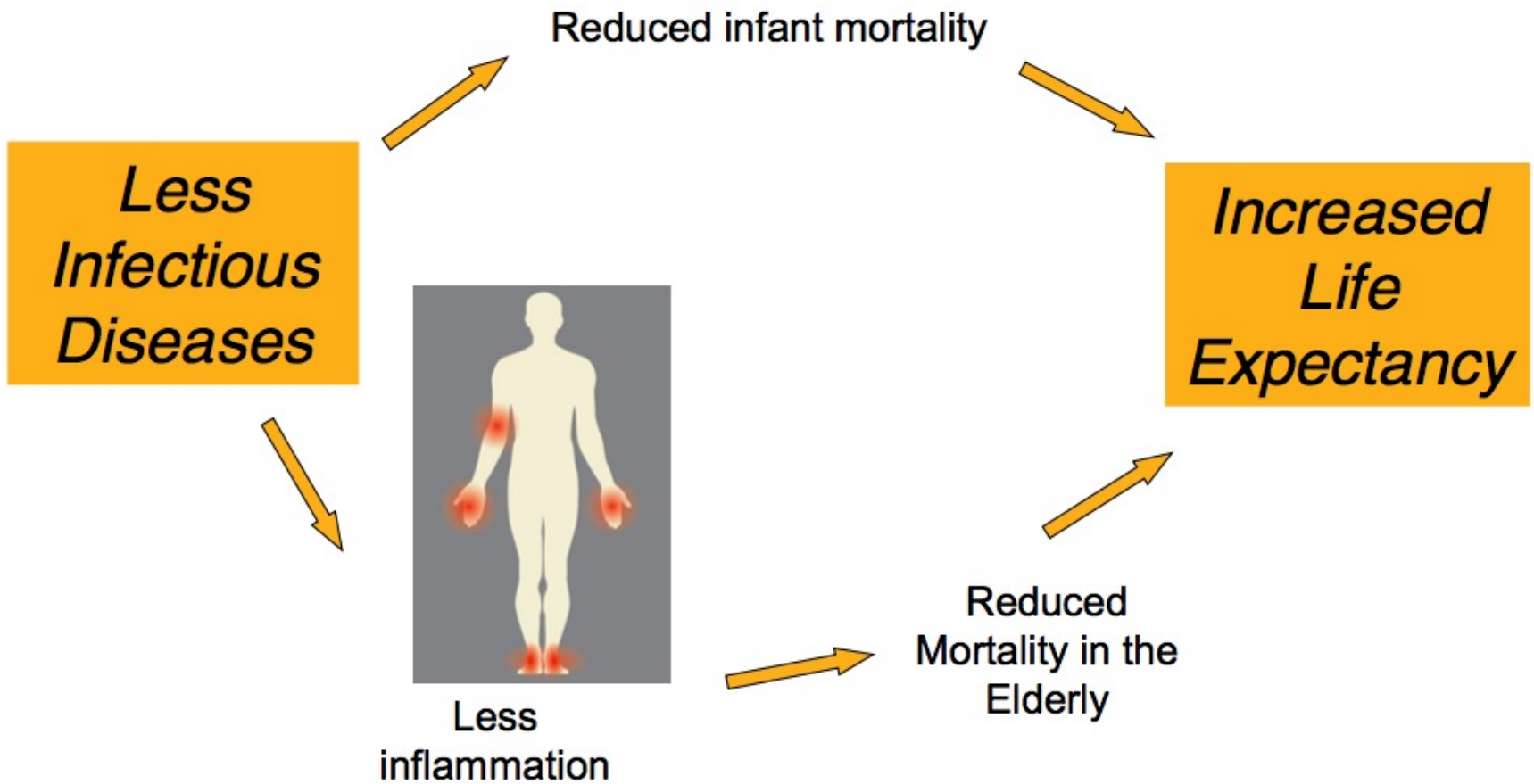
Producono gli anticorpi che si legano al patogeno estraneo per renderlo più visibile alle cellule che lo distruggeranno



Vaccination innovation, from 1880 to 2020

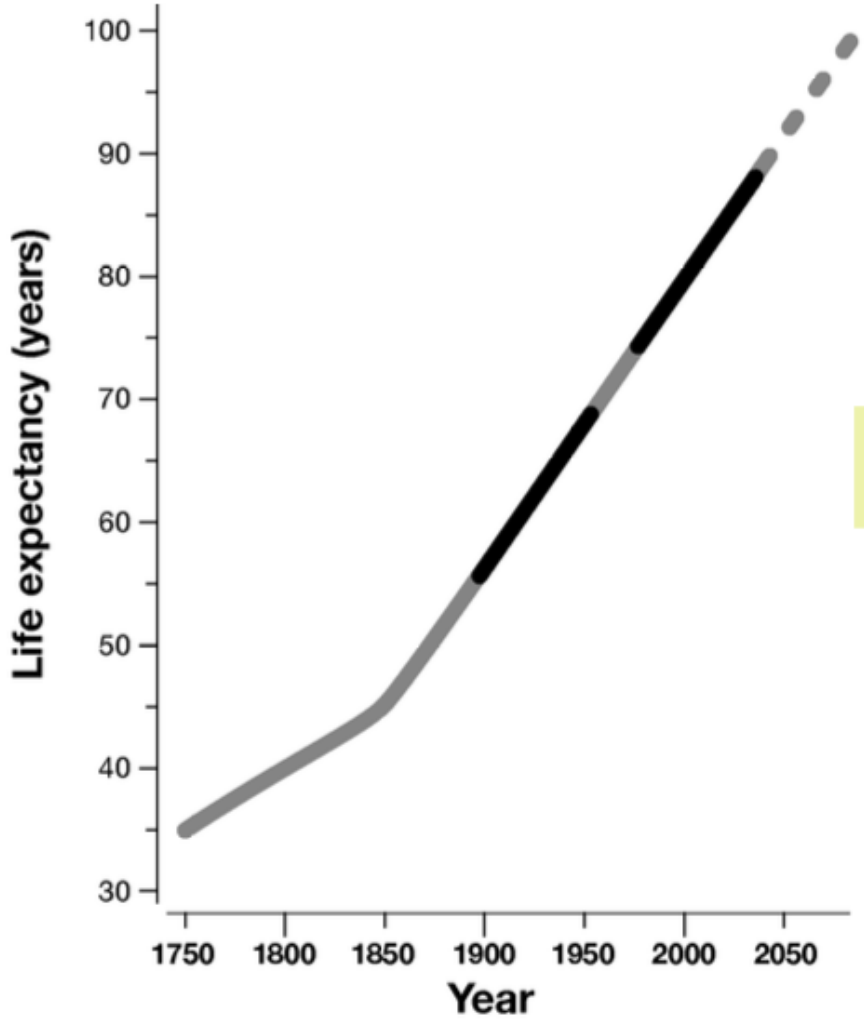


Crimmins et al. Attribute the Increase of Life Expectancy to the Conquest of Infectious Diseases



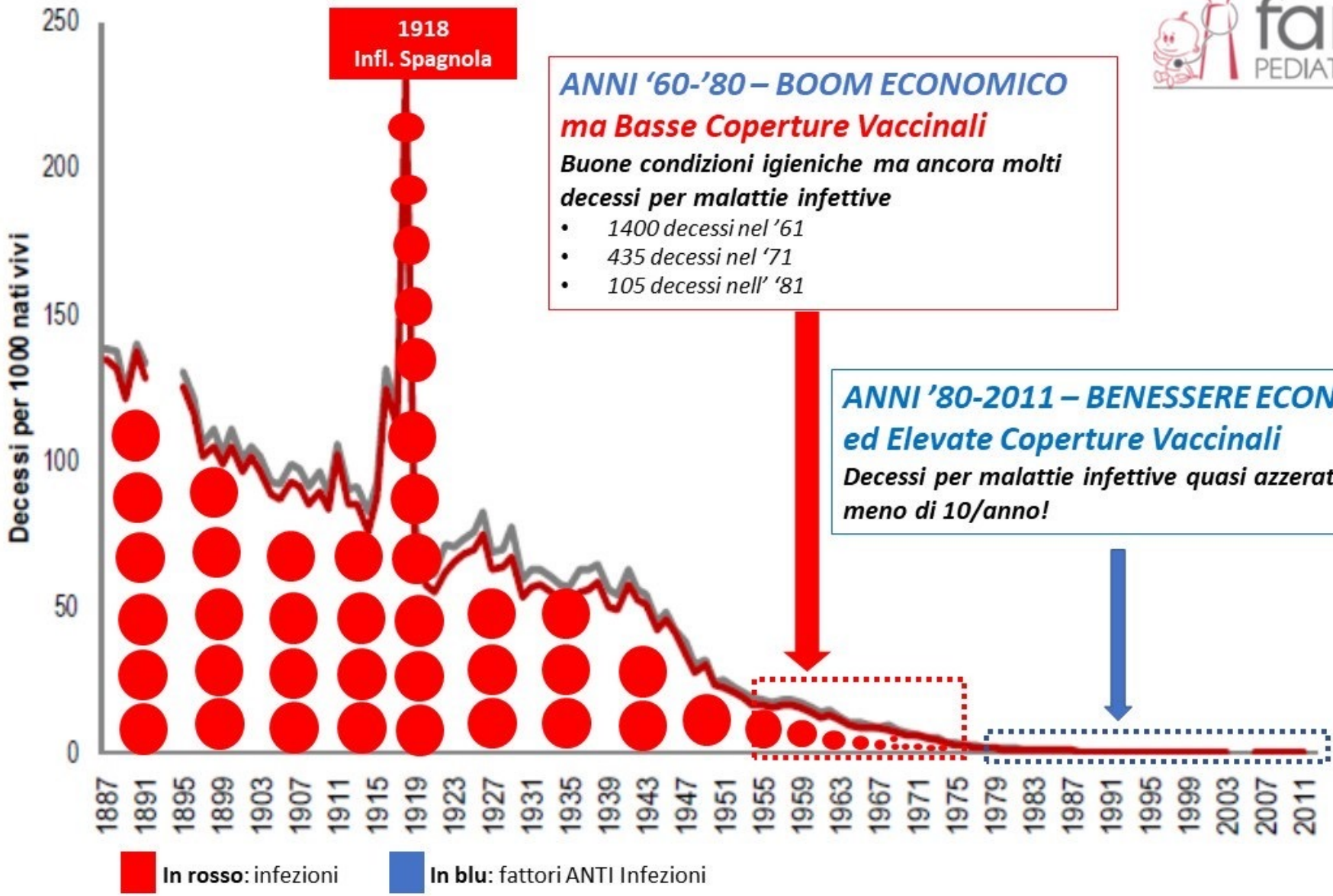
E. Crimmins & C. Finch. Infection, inflammation, height, and longevity. PNAS 103(2):498-503, 2006

people live longer



Which factors influenced this change?

TASSO DI MORTALITA' SOTTO I 5 ANNI IN ITALIA DAL 1887 AL 2011 PER MALATTIE INFETTIVE



ANNI '60-'80 – BOOM ECONOMICO
ma Basse Coperture Vaccinali

Buone condizioni igieniche ma ancora molti decessi per malattie infettive

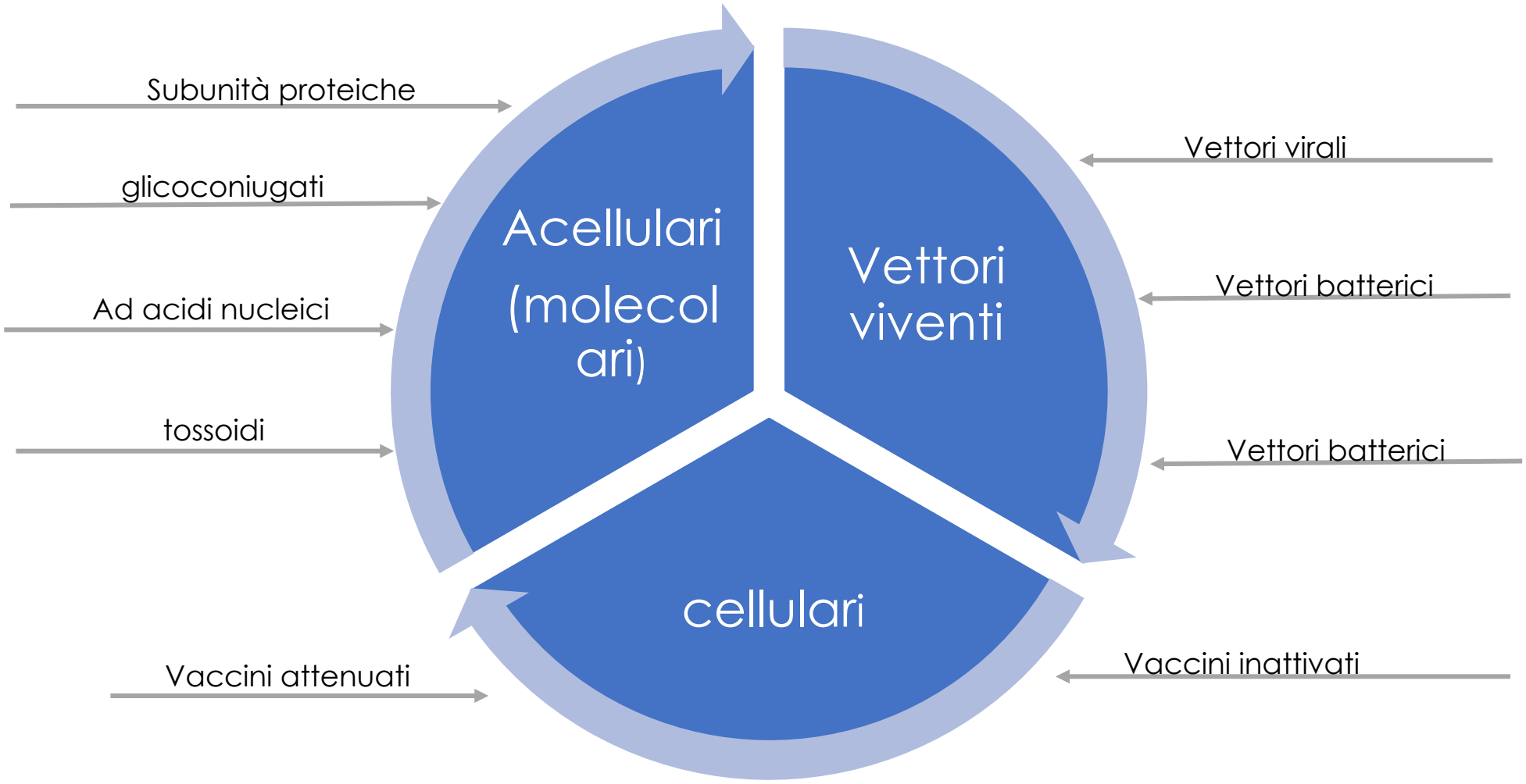
- *1400 decessi nel '61*
- *435 decessi nel '71*
- *105 decessi nell' '81*

ANNI '80-2011 – BENESSERE ECONOM. ed Elevate Coperture Vaccinali

Decessi per malattie infettive quasi azzerati - meno di 10/anno!

Focus ISTAT 15 Gennaio 2014 – Rielaborato da dr. R. Troiano per Faropediatrico.com

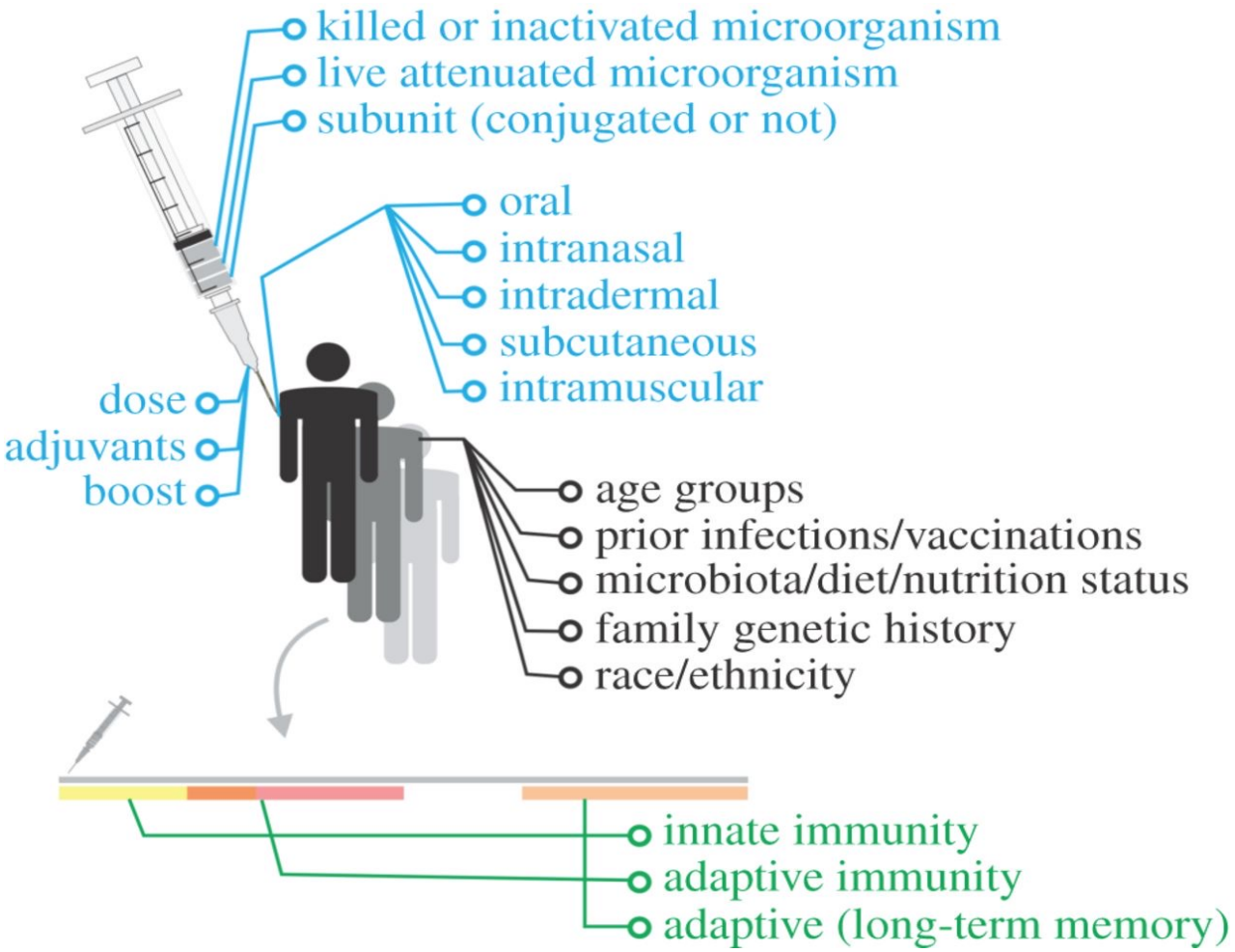
TIPOLOGIA DEI VACCINI



TIPOLOGIA DEI VACCINI

vaccini attenuati	vaccini inattivati	Vaccini a subunità	Vaccini ad acidi nucleici	Tossoide	Vettori vaccinali
• Polio orale (OPV) • Morbillo • Rotavirus • Febbre gialla	• Pertosse (cellulare) • Polio virus inattivato (IPV)	• Pertosse acellulare • Meningococco B (coniugato, MeningococcoC • Pneumococco (coniugato) • Epatite B	• a DNA • a RNA	• Tossoide tetanico • Tossoide Difterico	• Vettori virali • Vettori batterici • Vettori cellulari (vaccini per il cancro)

TIPOLOGIA DEI VACCINI e VACCINAZIONI



I vaccini interi inattivati

Pertosse (whole cell wP)

Poliomielite (IPV)

Generalità

Patogeno (batterio o virus) inattivati attraverso un processo fisico o chimico in condizioni di laboratorio

Risposta immunitaria

- Non sempre inducono una risposta immunitaria
- Risposta immunitaria non sempre permanente
- Possono essere richieste diverse iniezioni
- Necessario l'uso di
  - un'adiuvante

Sicurezza e stabilità

- Non inducono la malattia
- Più stabili rispetto ai vaccino viventi