If you can avoid an infectious disease, you better do…

Vaccines

Strategies to control infectious disease

- Living conditions and habits
  - Water purification (waterborn disease – 2 mil. death/year from diarrhoea)
  - sewage disposal (entteric infections)
  - improved nutrition (host defence – TB…)
  - better housing (less crowding, less dirt, but …. Allergies????)
- Food safety
  - Cold storage chain, hygiene of raw materials and treatment
  - Pasteurization (milk, cheese, cans etc.
  - food inspection (meat), preventing contamination (vegetables, chicken etc.)
  - proper cooking
- Vector and reservoir control
  - Control of arthropods – mosquitos, ticks, lice etc..
  - Control of animals that are source of zoonoses (rabies, bovine and badger TB
- Treatment or prevention
  - Drugs for chemotherapy
  - Vaccines
- Miscellaneous
  - Reduced promiscouity, condoms, hygiene, WC, clean syringes, blood screening

Standing on the shoulders of giants

Knowledge helps
= vaccines

Toxins, attenuated or killed disease-causing microorganisms

→ stimulation of the host immune system

→ production of „protective“ antibodies

- prophylactic (to prevent or ameliorate the effect of a future infection)
- therapeutic (to treat a developed disease)

Standing on the shoulders of giants

Robert Koch

Albert Calmette

Camille Guérin

Vaccines – a bit of history…

Live attenuated organisms

1796 – E. Jenner, cow pox material used to protect against smallpox,

cow = vacca in latine >>> Vaccine

1885 – L. Pasteur (dried brain rabbies vaccine)

1927 – BCG – Bacillus Calmette-Guérin – *M. bovis* attenuated by passaging on media

Post world War II – Measles, Mumps, Rubella, Adenovirus, Varicella, Typhoid, cholera

1957 – live attenuated polio virus – Sabine tested on his own sons…

CR first in the world to achive polio eradication **– lead by Karel Raška at WHO in geneva**

Killed Whole organisms

1896 - Typhoid, Cholera, 1897 - Plague

1926 – pertussis 1936 – influenza 1938 - rickettsia

Post world War II – Polio, Rabies, encephalitis, hepatitis A

1960s-1970s – eradication of smallpox

Purified proteins or polysaccharides, recombinant vaccines

1923 – diptheria, 1927 tetanus vaccine (diphtheria toxoid;

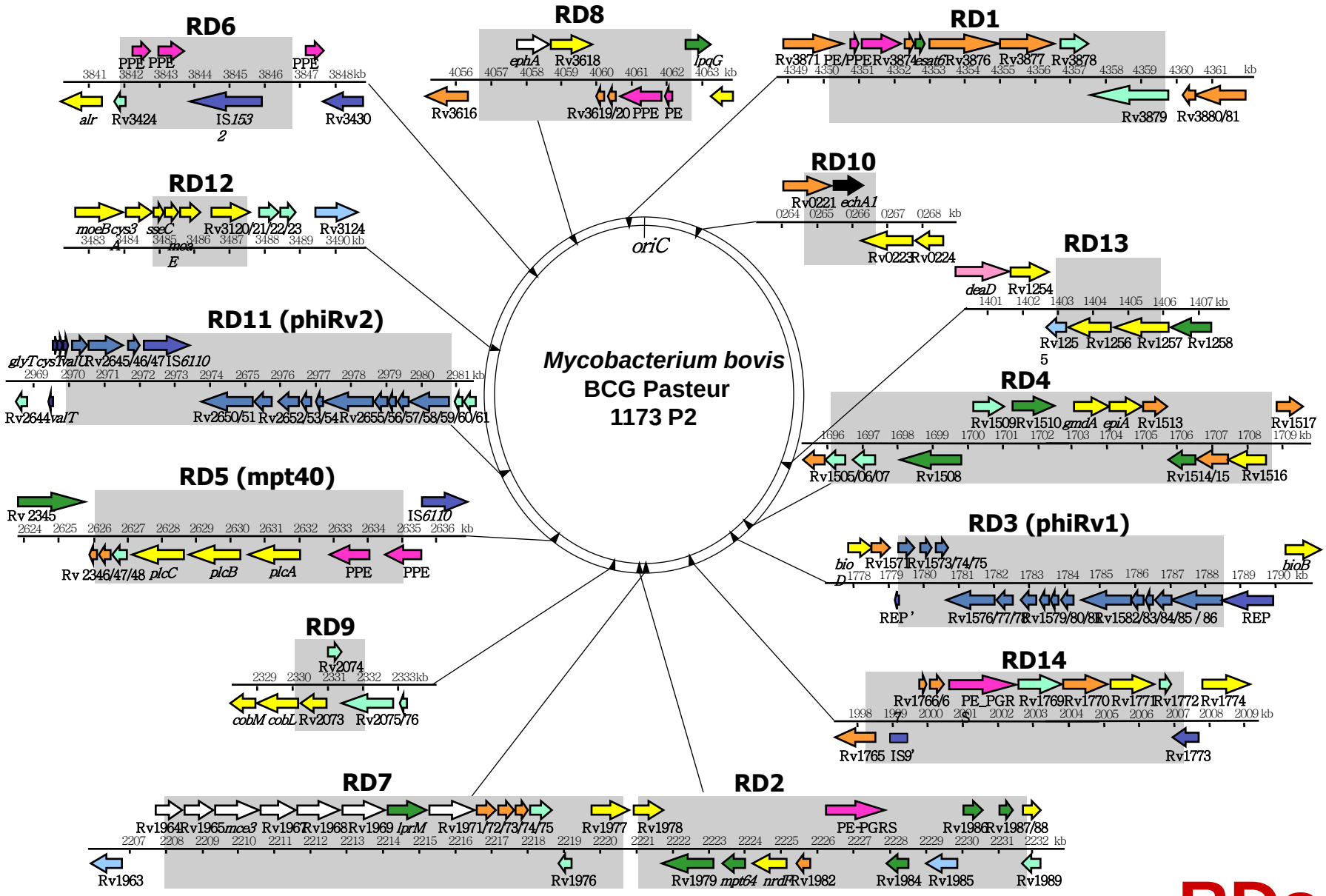
1940s DPT vaccine – first combination of diphteria and tetanus toxoids with *B. pertussis*)

1952 – J. Salk (inactivated polio vaccine)

, DNA vaccines

Vaccines remain elusive for many important diseases (HIV, malaria, even TB…) !

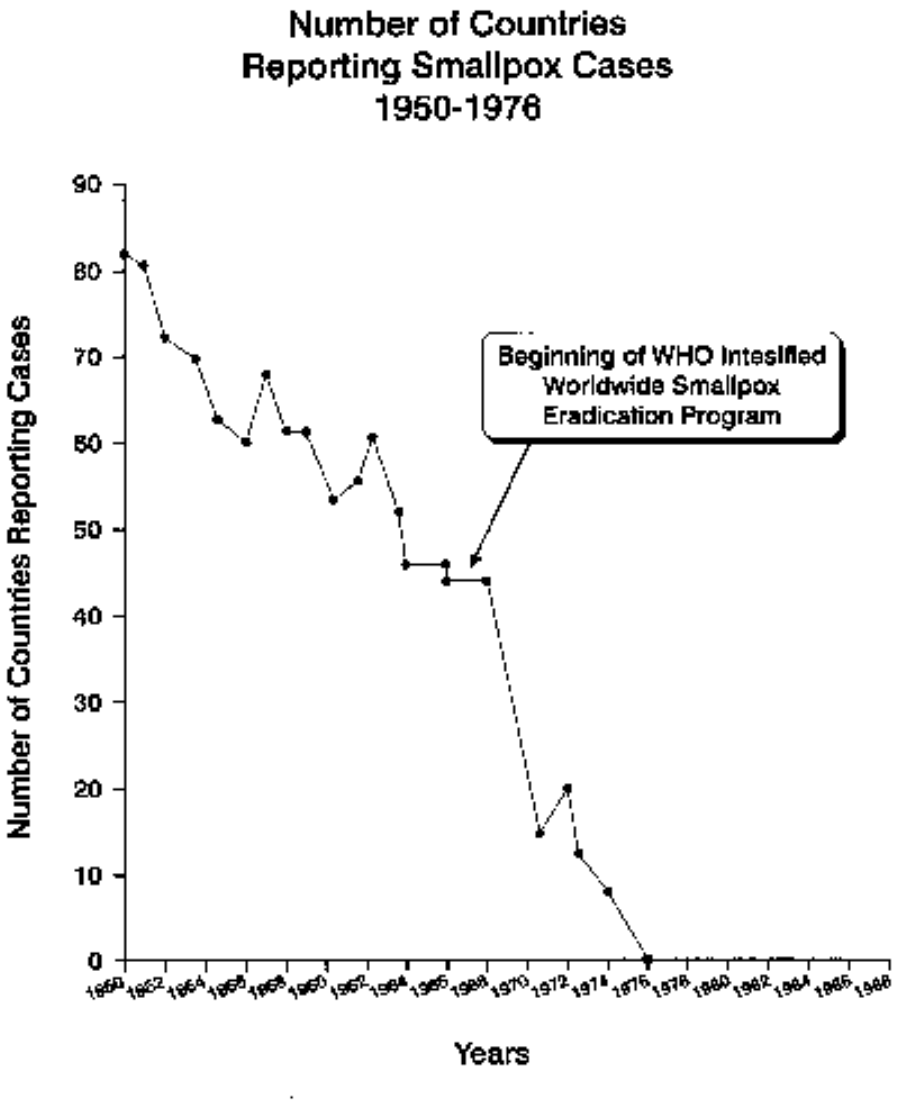
Major deleted regions in *Mycobacterium bovis* BCG Pasteur



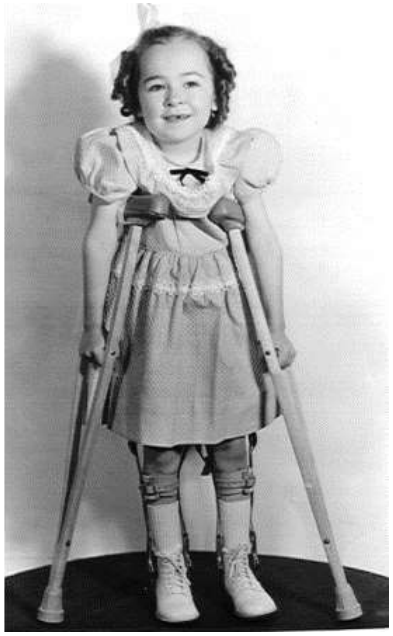
RDs

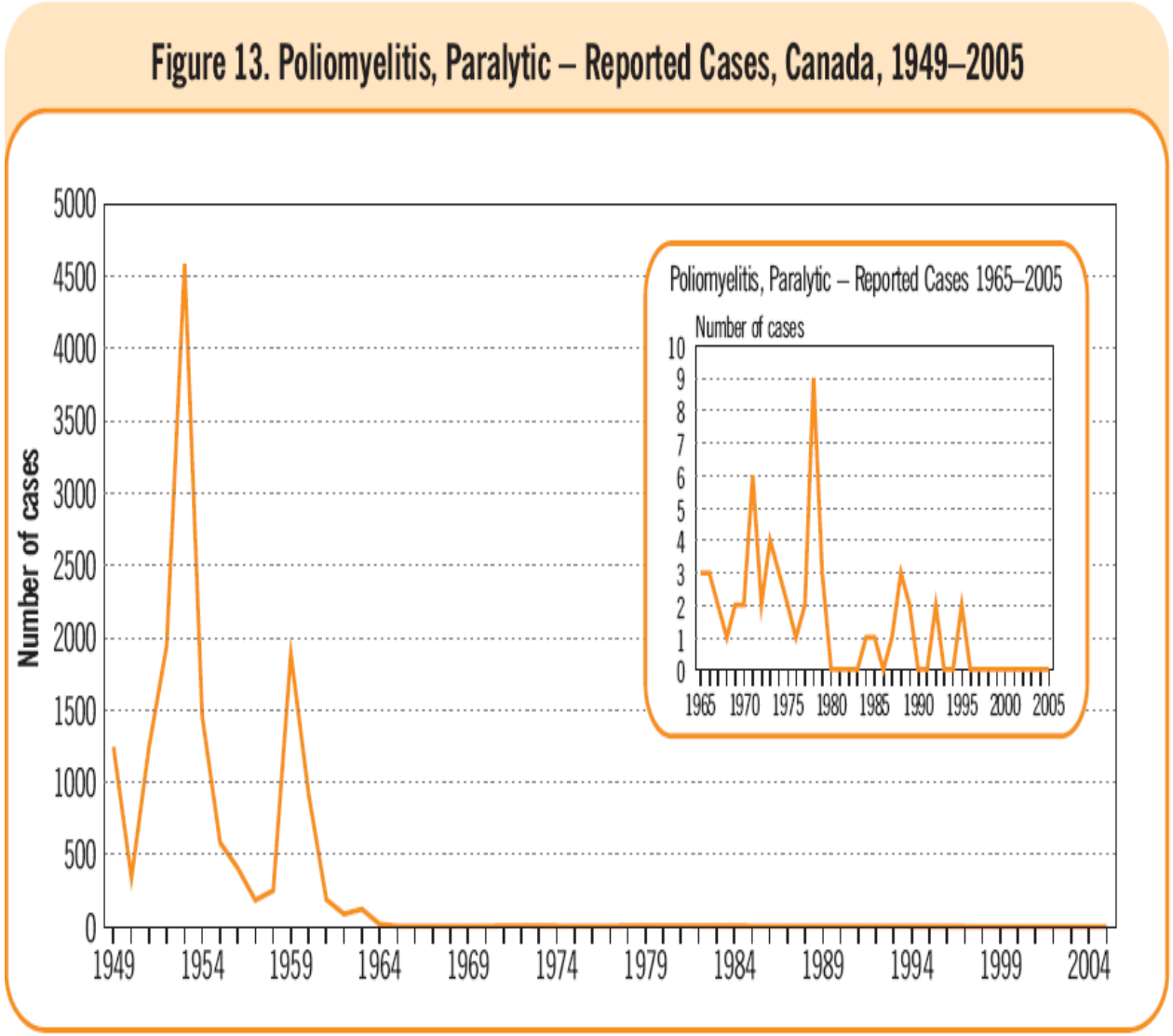
Unité de Génétique Moléculaire Bactérienne

The success story of vaccination



The success story of vaccination





Vakcína proti poliomyelitídě

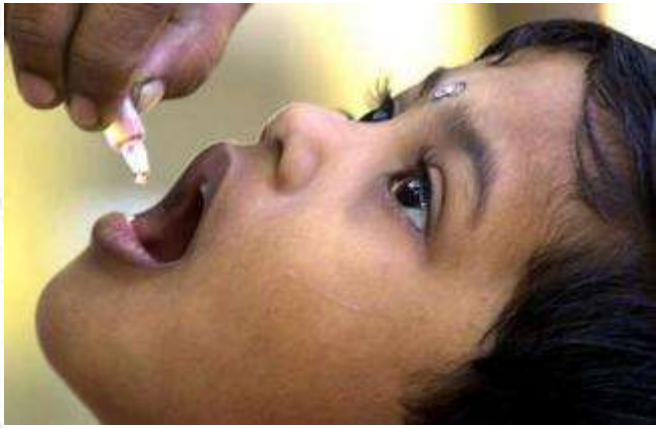
1955 inaktivovaná Salkova a 1960 atenuovaná Sabinova vakcína

Orální vakcína Alberta Sabina byla masivně testována v SSSR kolem roku 1960 Chumakovem a poté masově nasazena po celém světě v kampani SZO

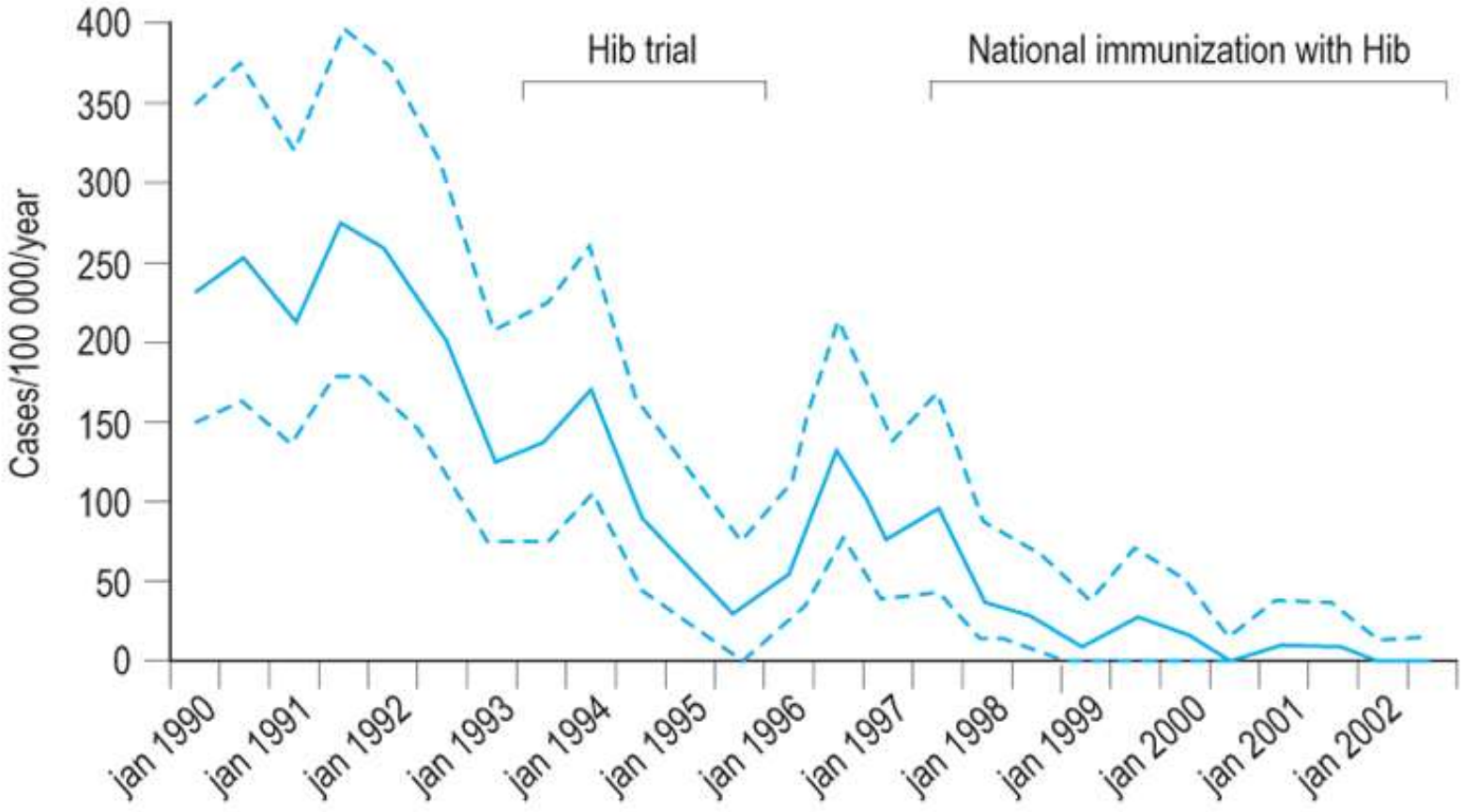
Československo se v roce 1961 stalo první zemí na světě, kde byl přerušen proces šíření divokých poliovirů v populaci a poliomyelitida zde byla eradikována vakcínou vyvinutou týmem doc. Slonima v ÚSOL Praha

Jonas Salk

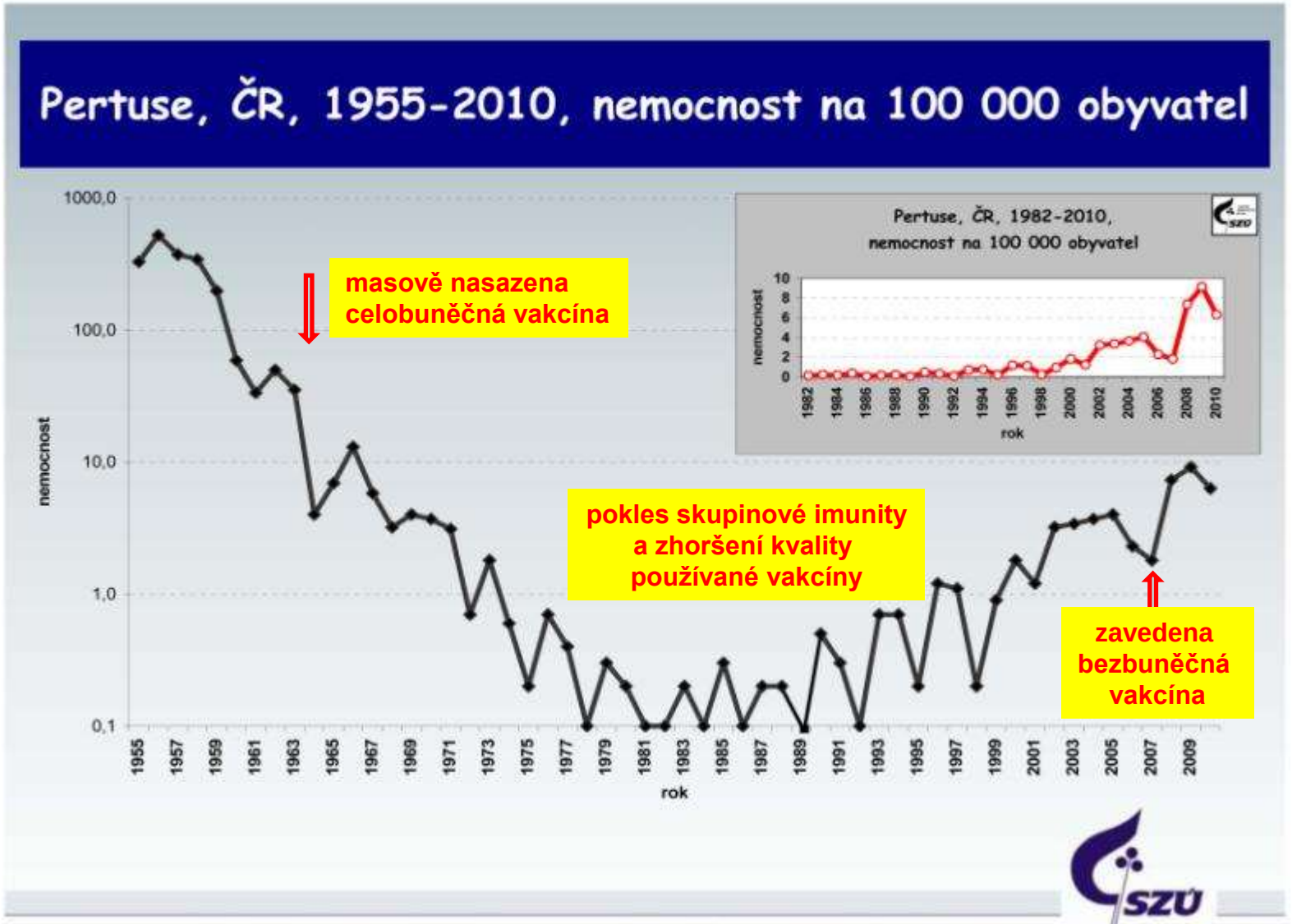
Albert Sabin



The success story of vaccination



Očkování inaktivovanou celobuněčnou pertusovou vakcínou bývalo velmi účinné…



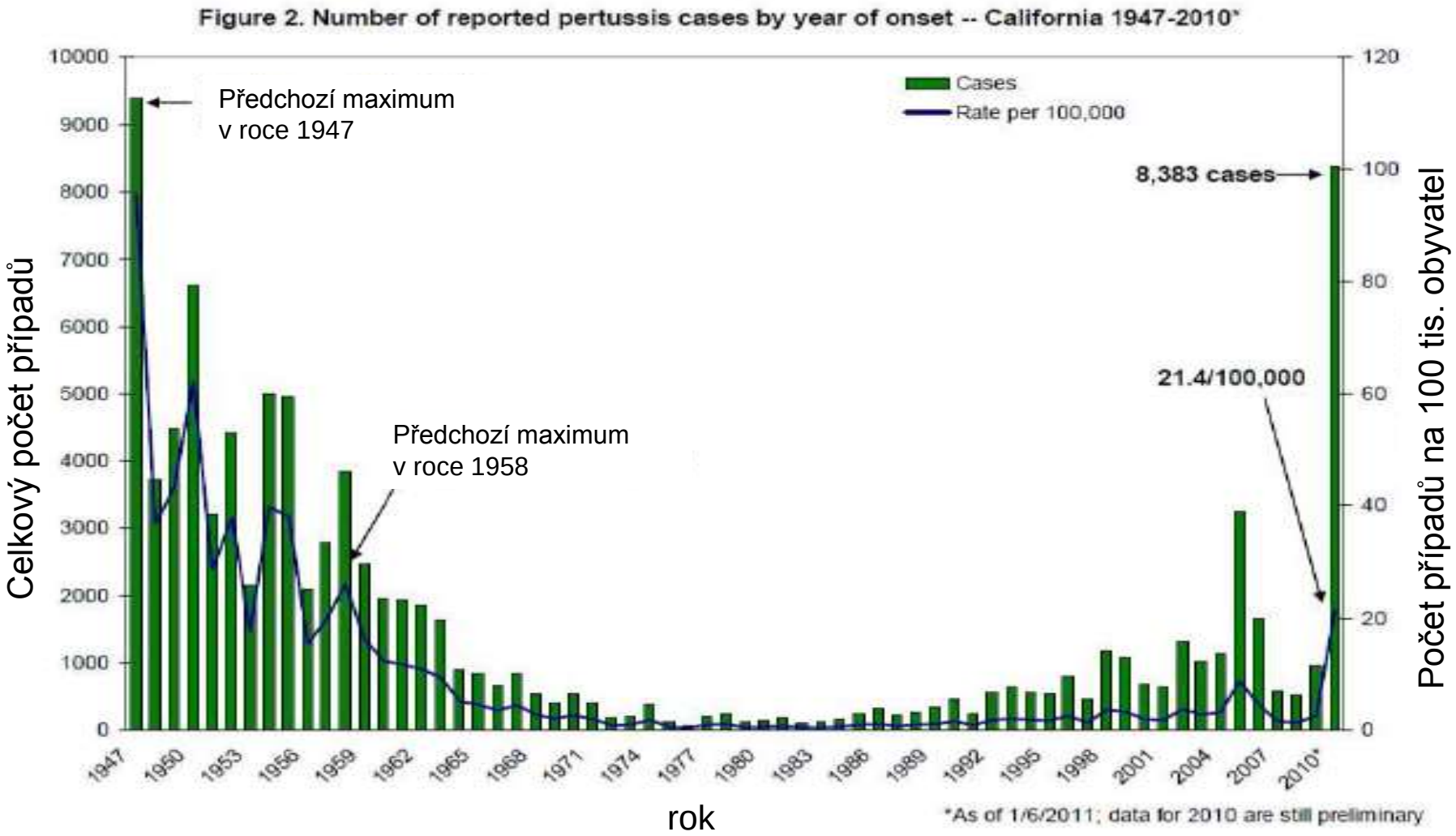
Díky “demokracii” a zavedení nové acelulární vakcíny se dávivý kašel vrací do nejvyspělejších zemí ….

V letech 2009-2012 proběhla v Austrálii epidemie dávivého kašle

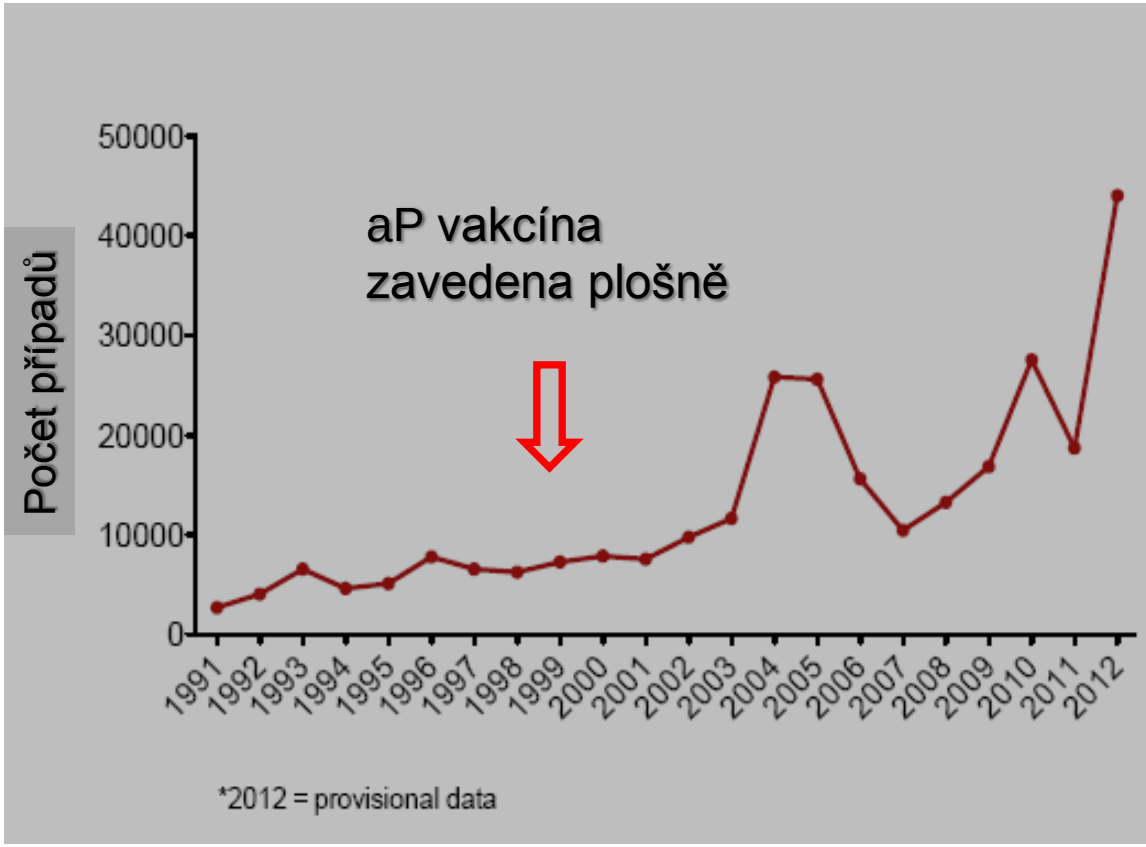
>100 případů / 100 tisíc obyvatel…

jako kdyby neočkovali vůbec…

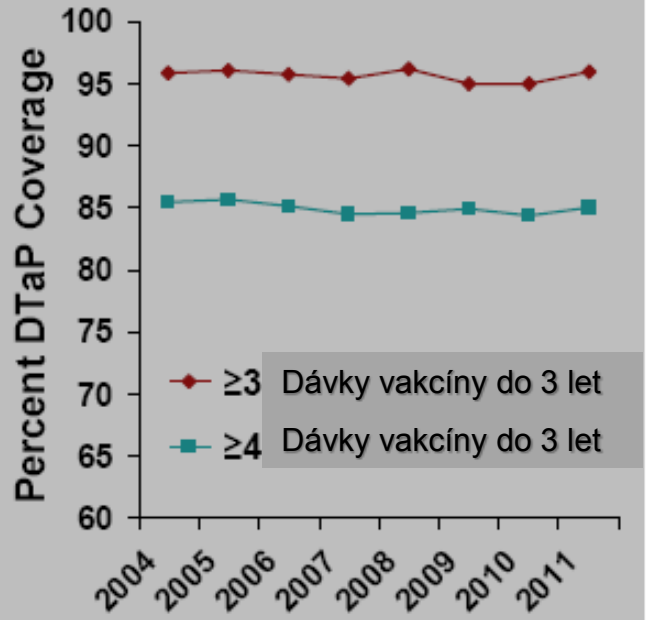
Epidemie dávivého kašle v Kalifornii v roce 2010 zemřelo 10 kojenců mladších než 2 měsíce



Návrat pertuse do USA v roce 2012 přes pokrytí 96% dětské populace acelulární vakcínou

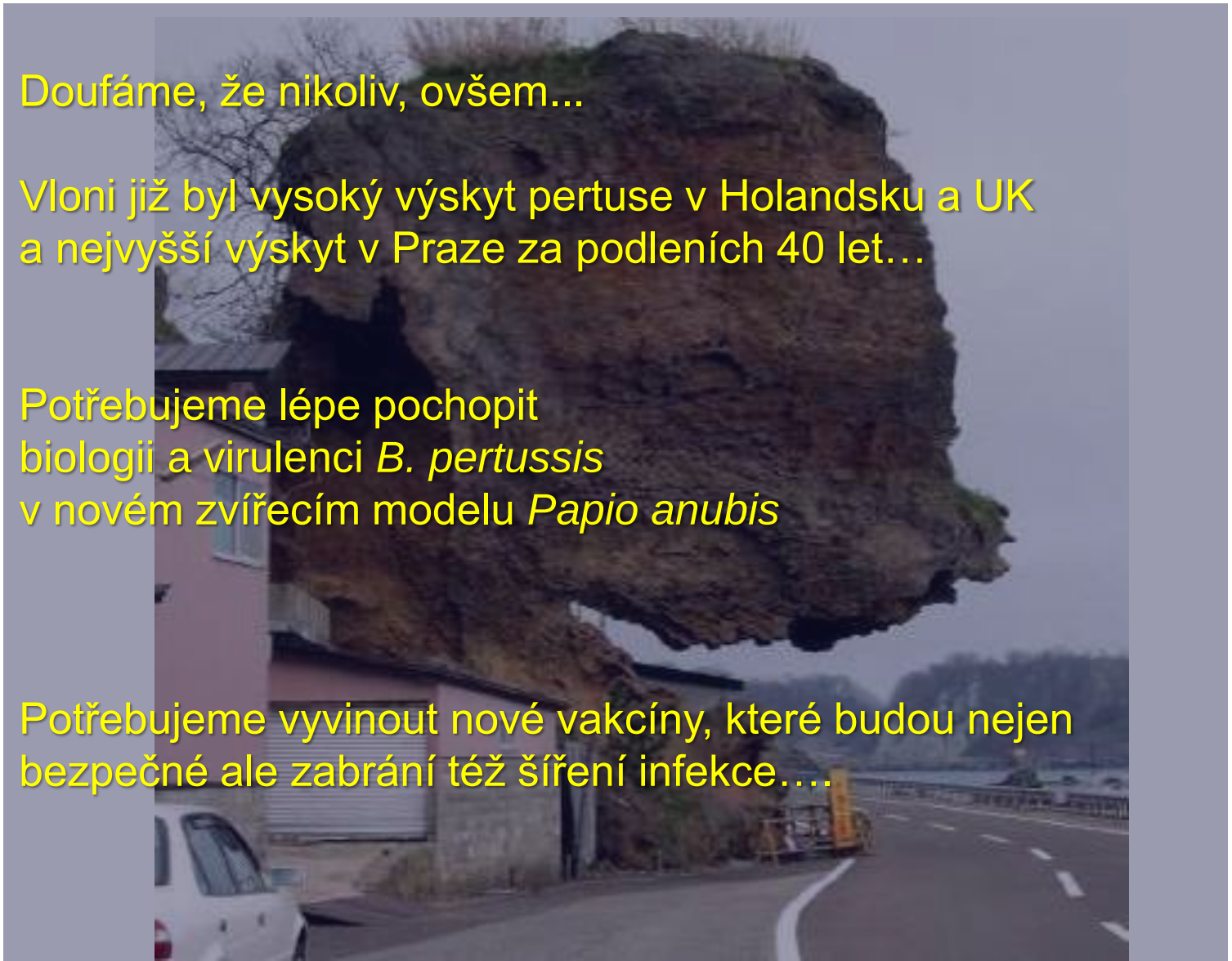


CDC National Notifiable Diseases Surveillance System



National Immunization Survey
Pertussis Surveillance System

Představuje pertuse tohle?



Doufáme, že nikoliv, ovšem...

Vloni již byl vysoký výskyt pertuse v Holandsku a UK
a nejvyšší výskyt v Praze za podleních 40 let…

Potřebujeme lépe pochopit
biologii a virulenci *B. pertussis*
v novém zvířecím modelu *Papio anubis*

Potřebujeme vyvinout nové vakcíny, které budou nejen
bezpečné ale zabrání též šíření infekce….

Klasické vakcíny předběhly poznání imunitního systému:

- Připraveny čistě empiricky z celých bakteriálních buněk a virů
- **Dodnes patří k nejúčinnějším vakcínám díky spektru obsažených antigenů**

- Jistá míra vedlejších účinků vedlejších a mnohdy zveličovaných účinků (obsah endotoxinu, reverze poliovirů, automunita?).

Individuální bezpečnost na úkor ochrany populace???

Aktuální výzvy ve vývoji vakcín

- Malárie - *P. falciparum* – *nová generace vakcín v klinických testech*
- Tuberkulóza - 2 mld. latentně infikovaných – běží klinické testy
- AIDS – 35 milionů HIV-pozitivních – *zatím beznadějné?*
- *Chlamydia pneumoniae*
- infekční mononukleóza – EBV a též CMV

.....

- Imunoterapeutické vakcíny proti alergiím, autoimunitě
- T-buněčné vakcíny pro imunoterapii rakoviny

slibné, první vakcína na léčbu nádorů prostaty registrovaná v USA

Nové vakcíny staví na pokroku molekulární biologie, genomiky a buněčné imunologie

Bacterial subunit vaccines - SEARCH FOR VIRULENCE – ASSOCIATED GENES

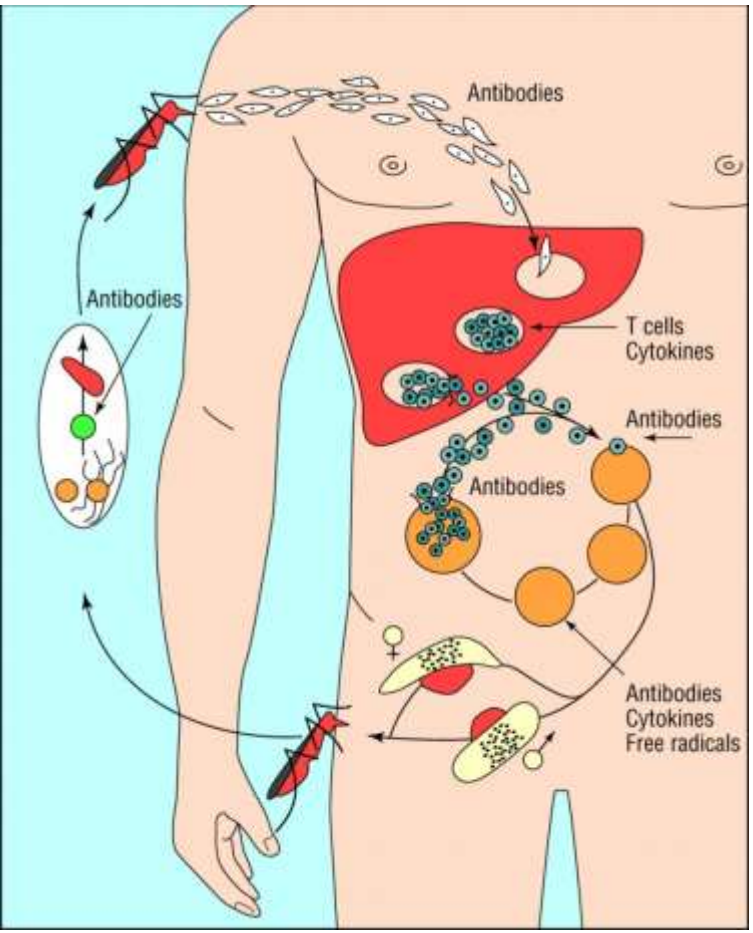
- Mutagenesis (disruption of virulence genes)
- Allelic exchange (gene replacement)
- Complementation analysis (gene function restorement)
- Operon fusions (regulon mapping)
- Secreted proteins
- In vivo during infection specificaly activated genes:
  - IVET
  - differential display PCR
  - substractive hybridization
  - signature tag mutagenesis with negative selection
- Two-hybrid analysis, phage display (interactive gene products identification)
- Pathogenicity islands
- Whole genom sequencing + selective mutagenesis (virulence tests)

! Mechanism of pathogenesis, antigenicity vs. immunogenicity, antigen variability !

Nové koncepty (1):

- Nová adjuvans – soli hliníku, MPL, olejové suspenze PolyICLC – aktivují inflamasom anebo signalizují přes TLR ligandy (poly I:C + polylysin)
- konjugace málo imunogenního kapsulárního polysacharidu s proteiny (*Hemophilus*, *Neisseria meningitidis* C, *Streptococcus pneumonieae)*
- rekombinantní pseudovirové částice (HPV, HBV)
- orální a intranasální atenuované a enkapsulované vakcíny (rotavirus, *Salmonella, Shigella*, poxvirus, adenovirus, intranasální chřipková vakcína)
- rekombinantní podjednotky vakcín, např. gdPT použitý v italské vakcíně

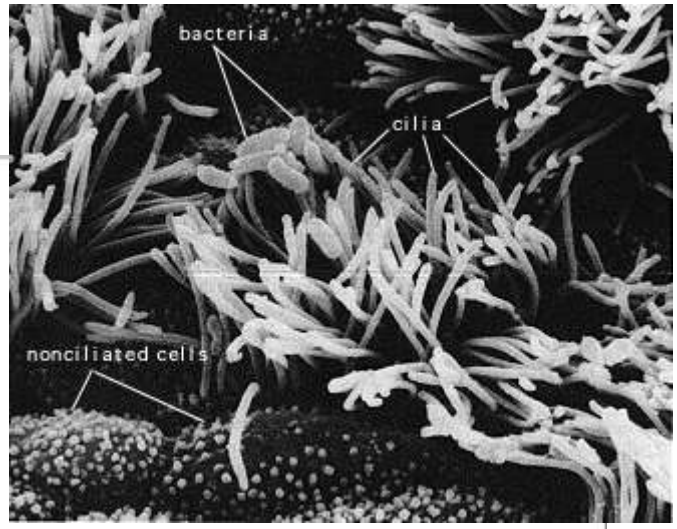
The issue of malaria vaccine



stage	vaccine strategy	vaccine candidates
sporozoites	sporozoite vaccine to induce blocking antibody, already field-tested in humans	circumsporozoite protein (RTS,S) thrombospondin - related adhesive protein (TRAP)
liver stage	sporozoite vaccine to induce cell-mediated immunity to liver stage, already tested in humans	
merozoites	merozoite (antigen) vaccine to induce invasion inhibitory antibody	merozoite surface protein - 1 (MSP1) apical membrane antigen - 1 (APA-1)
asexual erythrocyte stage	asexual stage (antigen) vaccine to induce other responses to red cell stage, and against toxic products ('anti-disease' vaccine)	glutamate - rich protein (GLURP)
gametocytes	vaccines to interrupt sexual stages – 'transmission blocking' vaccine	
gametes		

VACCINE TYPES

-**Killed** (previously virulent microorganisms destroyed with chemicals or heat)



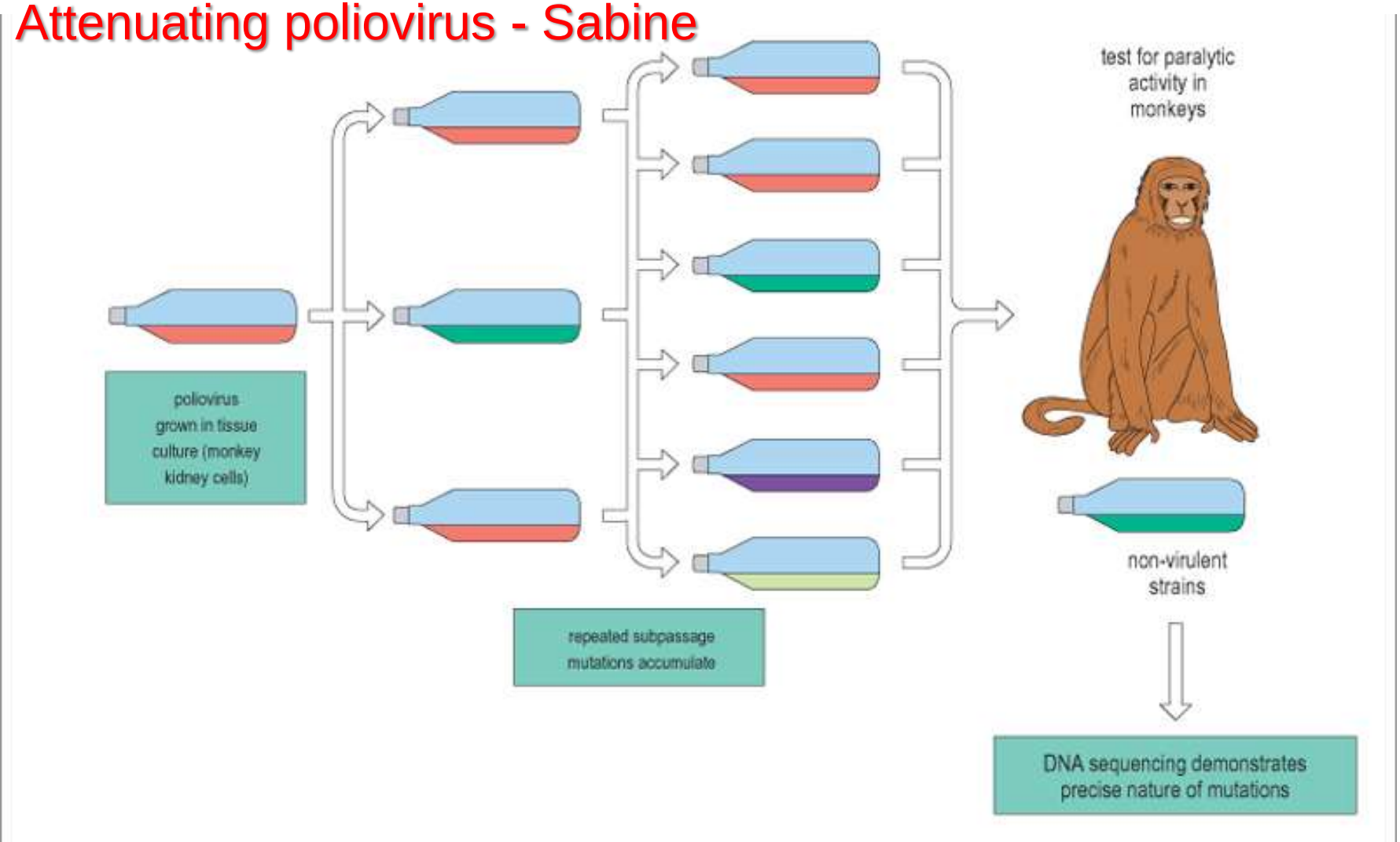
Thanks to democracy and acellular vaccine, pertussis is coming back…

This spring already 9 deaths and 600 seriously ill infants in California …

VACCINE TYPES

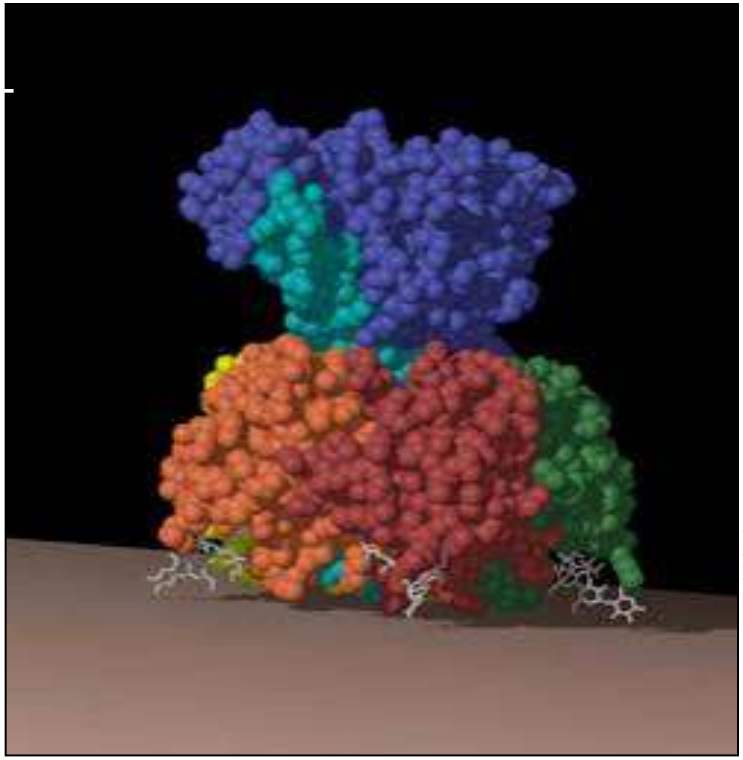
- **Attenuated** (live „weakened“ microorganisms), preferred type for vaccination of healthy adults. includes for ex. viral diseases: yellow fever, measles, rubeolla, mumps; and bacterial tuberculosis (modified strain BCG is used to elicit immunogenicity tothe vaccine)

Attenuating poliovirus - Sabine

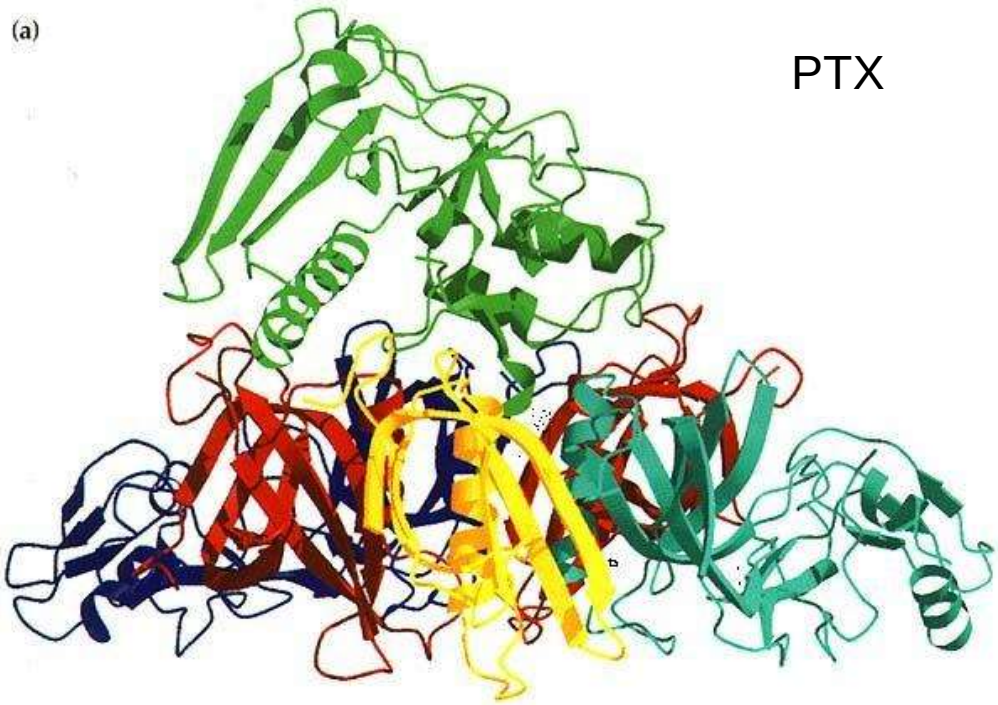


VACCINE TYPES

- **Toxoid** (chemically of genetically inactivated toxic



Merritt *et al.*, Prot. Sci. 3: 166-175, 1994



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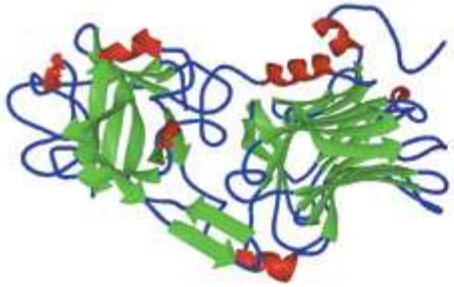
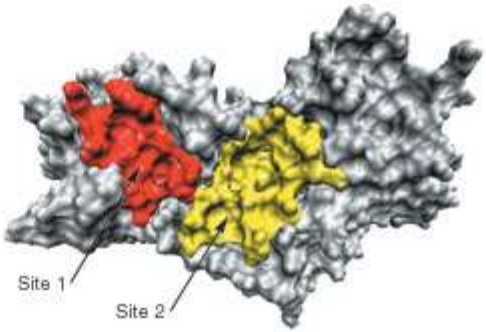
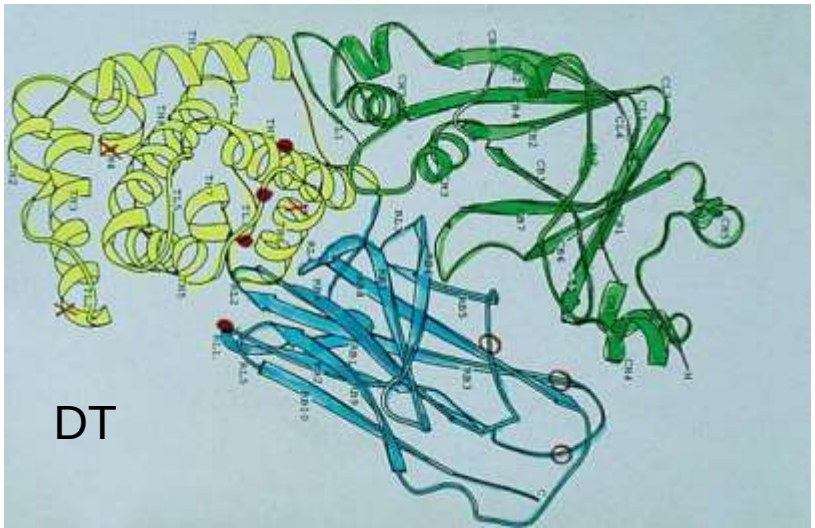
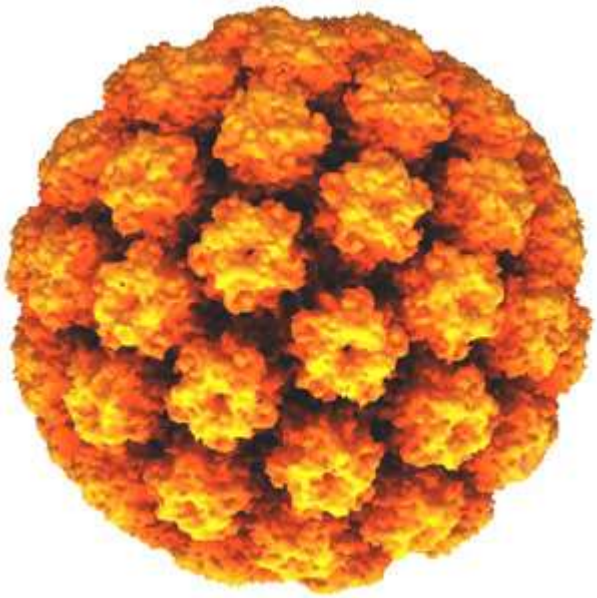






VACCINE TYPES

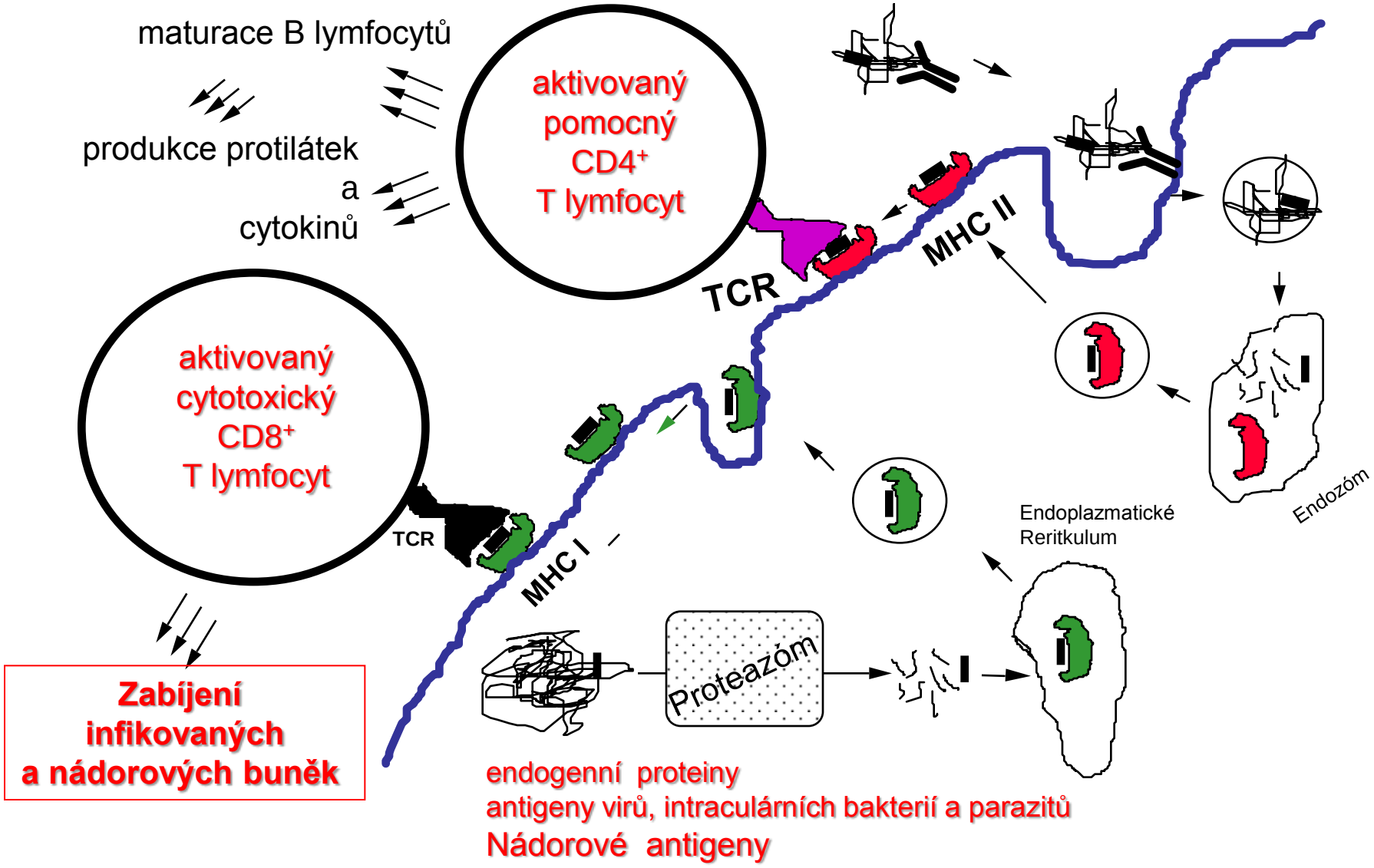
- **Subunit** (protein subunit; hepatitis B virus envelope proteins recombinantly produced in yeast, virus-like particle against human papilloma virus or HA and NEU subunits of the influenza virus)



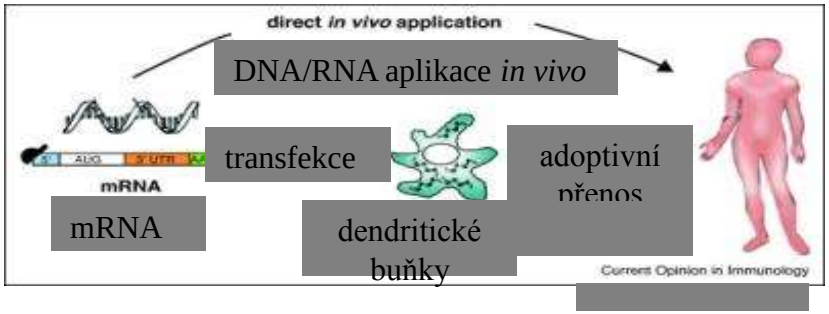
VLP of HPV16

T-buněčné vakcíny pro prevenci infekcí a terapii nádorů

Dvě dráhy prezentace antigenů: cesta k protinádorovým vakcínám



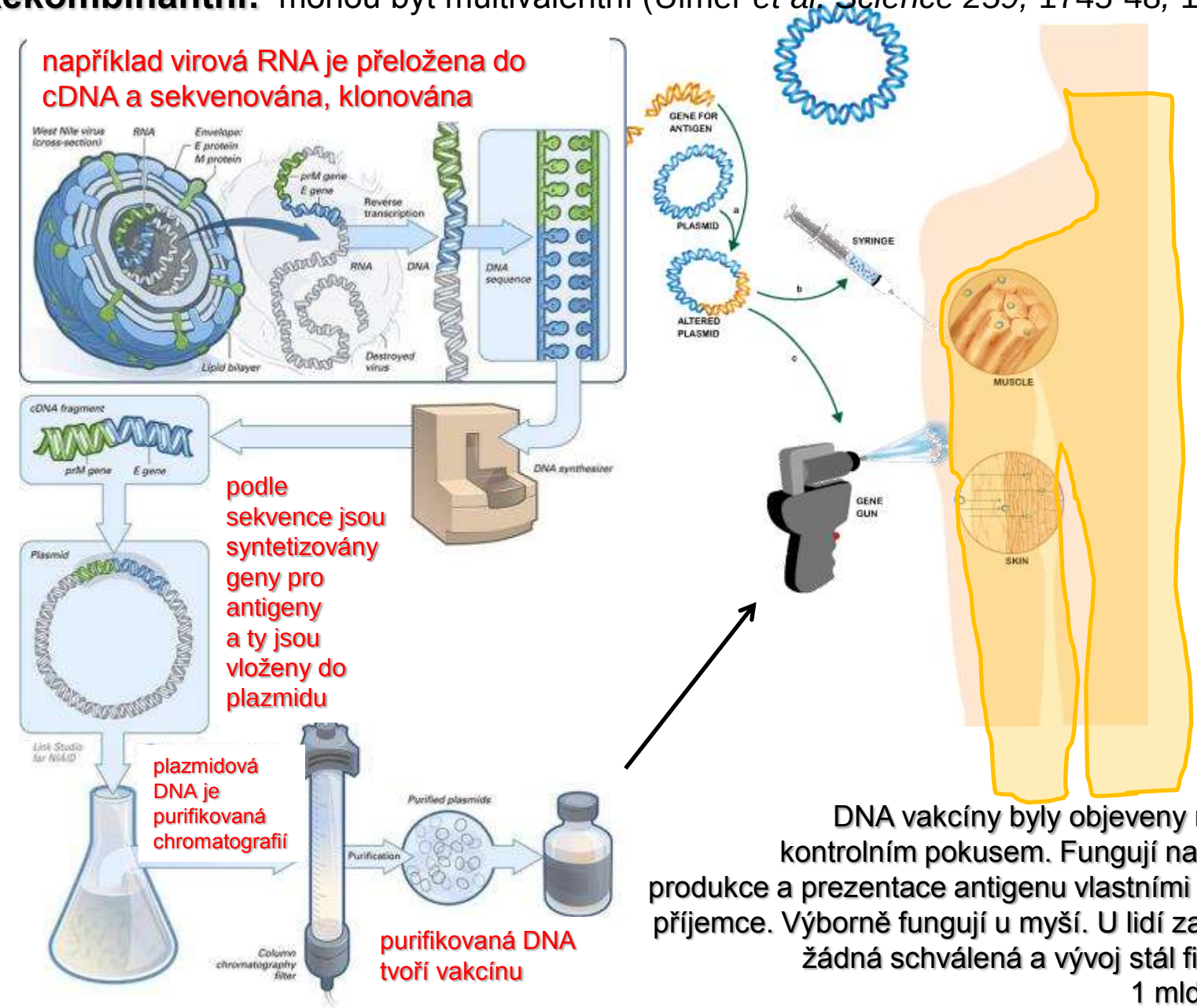
Jak dopravit antigen **do cytosolu** prezentujících buněk a stimulovat $CD8^+$ cytoxické T lymfocyty?



- DNA a RNA vakcíny – i.d./s.c./i.m. nebo *ex vivo* aplikace na APC a transfer do těla
  (zatím jenom pro zvířata)
- dendritické buňky maturované a nabité antigeny *in vitro* pro imunoterapii nádorů (Provenge = první registrovaná vakcína na rakovinu prostaty – 2010)
- Intracelulární baktérie - uvolňující rekombinantní antigeny (atenuovaná *Listerie, Salmonella, BCG…)*
- Atenuované rekombinantní viry produkující antigeny (např. vakcínie, adenovirus)
- Liposomové preparace peptidů
- **Proteiny pronikající do buněk (Tat, bakteriální toxoidy - dCyaA (Procervix)**
- protilátky s geneticky (nebo chemicky) připojeným antigenem, které rozeznávající endocytické receptory dendritických buněk (R. Steinman – Celldex Therapeutics)
  antigenní fuze se streptavidinem (v komplexu s biotinylovanou směřovací protilátkou?)

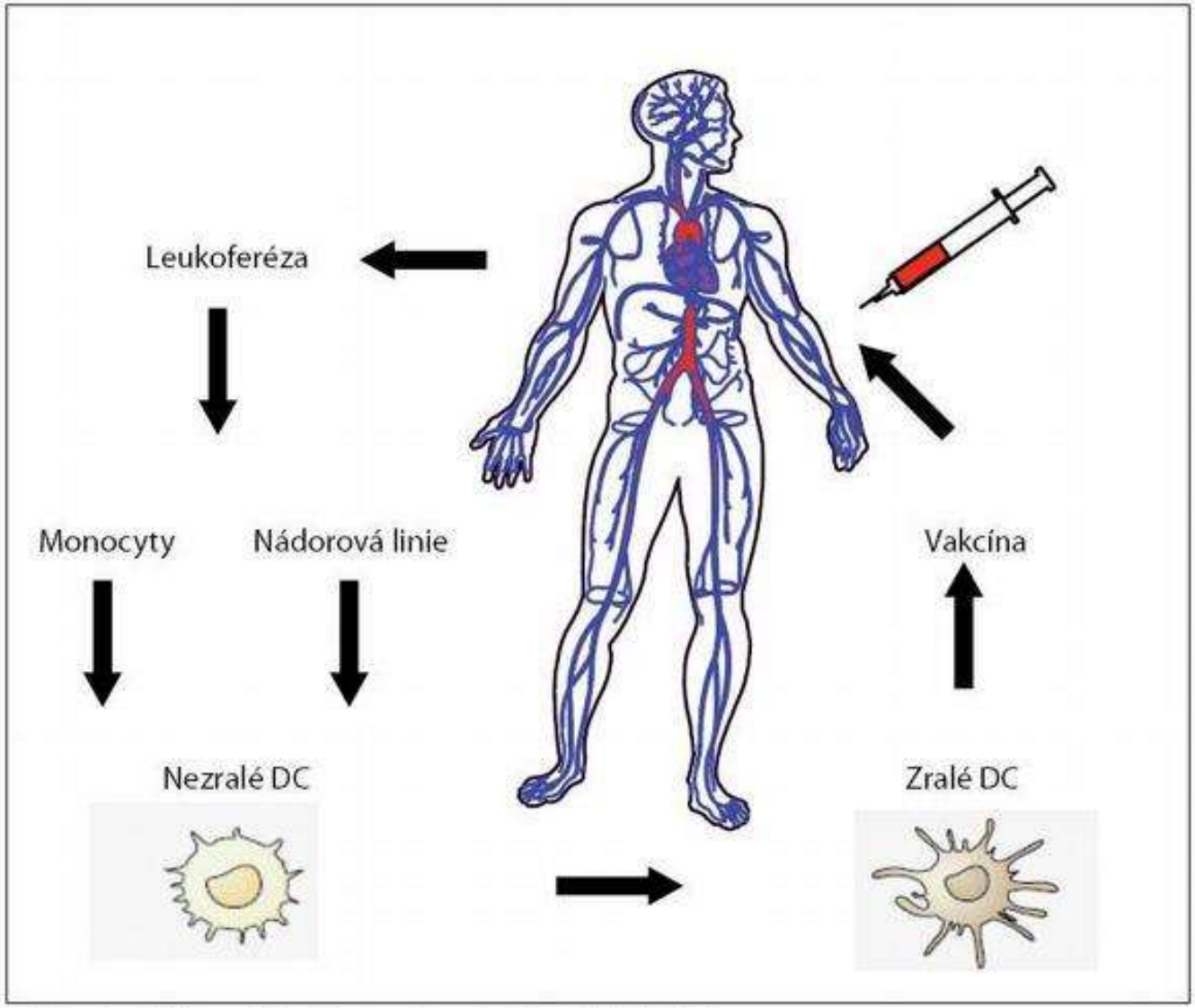
DNA vakcíny

Rekombinantní: mohou být multivalentní (Ulmer *et al. Science 259*, 1745-48, 1993)

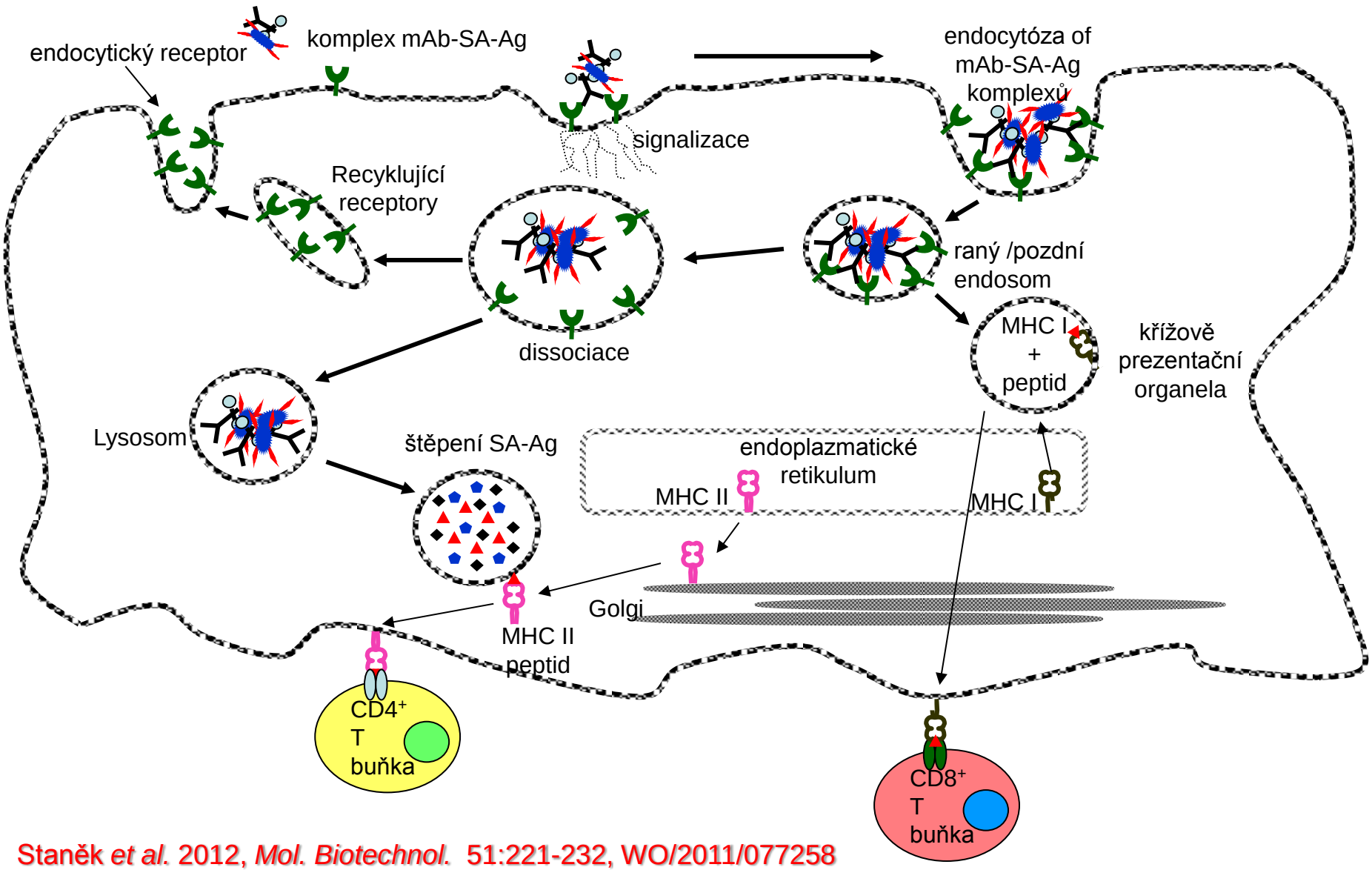


DNA vakcíny byly objeveny náhodou kontrolním pokusem. Fungují na principu produkce a prezentace antigenu vlastními buňkami příjemce. Výborně fungují u myší. U lidí zatím není žádná schválená a vývoj stál firmy cca. 1 mld. USD…

Dendritické buňky maturované a nabité antigeny z lyzátu nádorových buněk *in vitro*



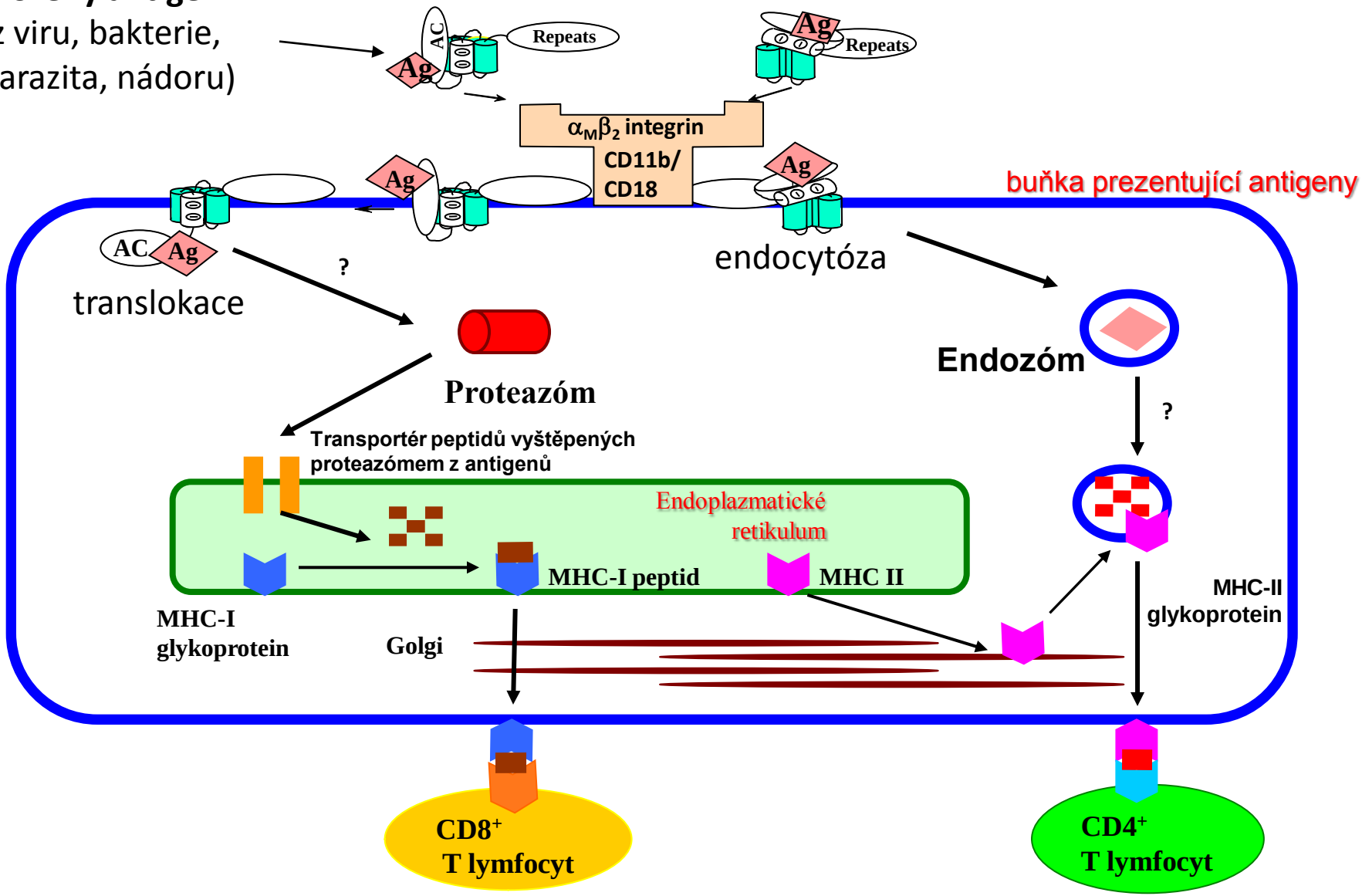
Využití antigenních fuzí se sreptavidinem pro dopravu antigenů ke prezentaci na MHC molekulách



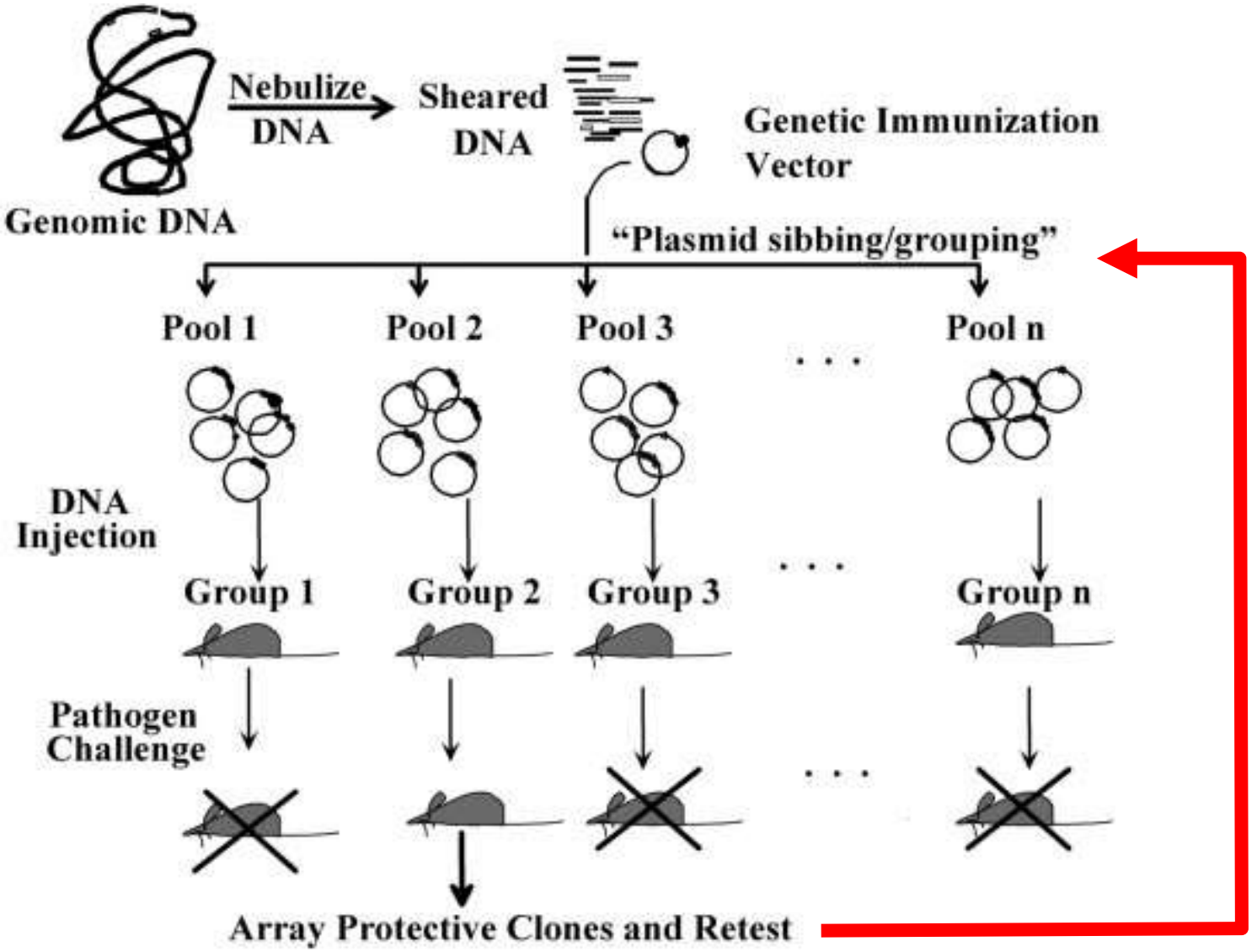
Staněk *et al.* 2012, *Mol. Biotechnol.* 51:221-232, WO/2011/077258

dCyaA toxoid jako nástroj pro dopravu antigenů do cytosolu prezentujících buněk

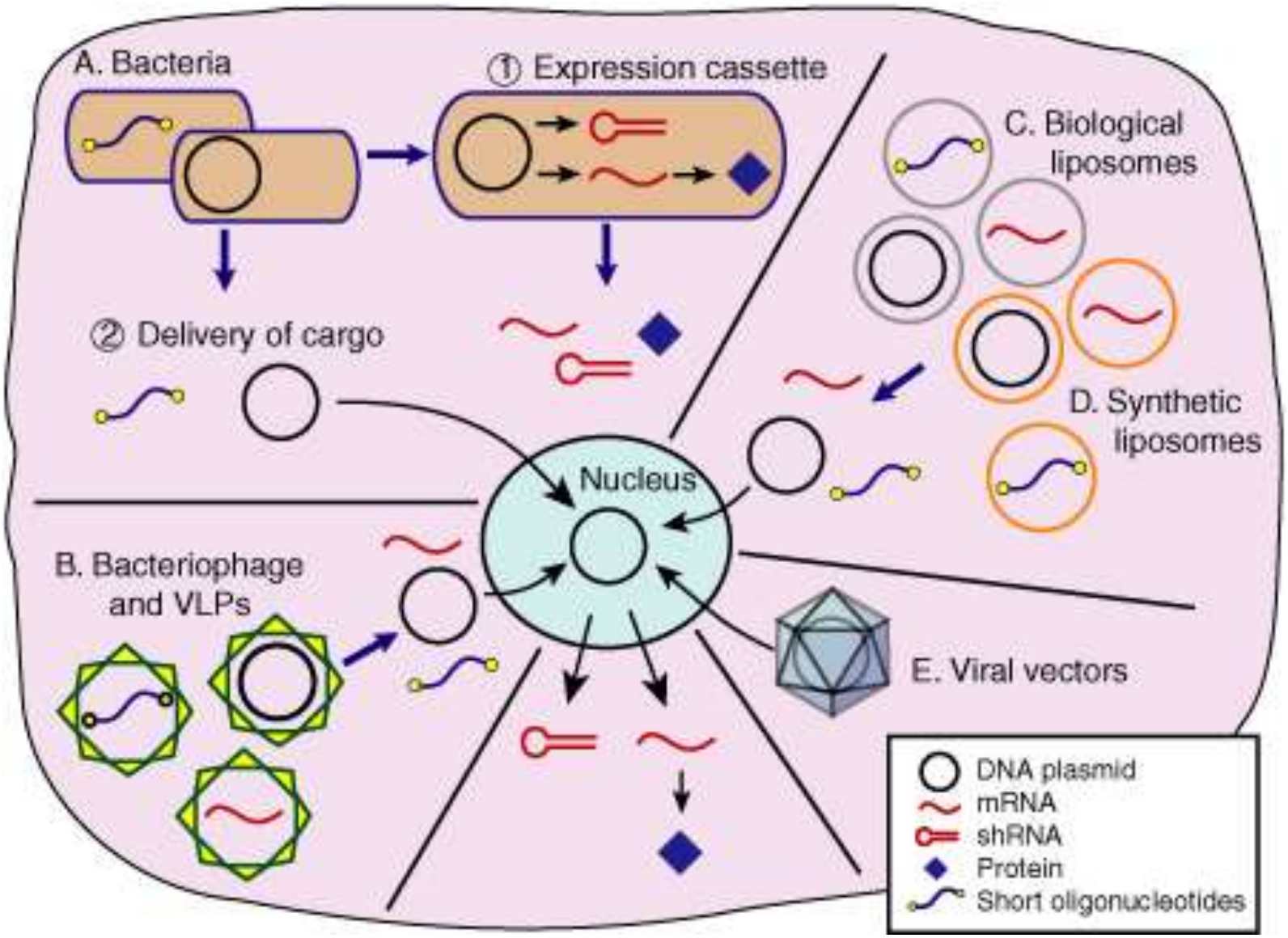
Vložený antigen
(z viru, bakterie, parazita, nádoru)



Genetic to genomic vaccination
- Expression library immunization



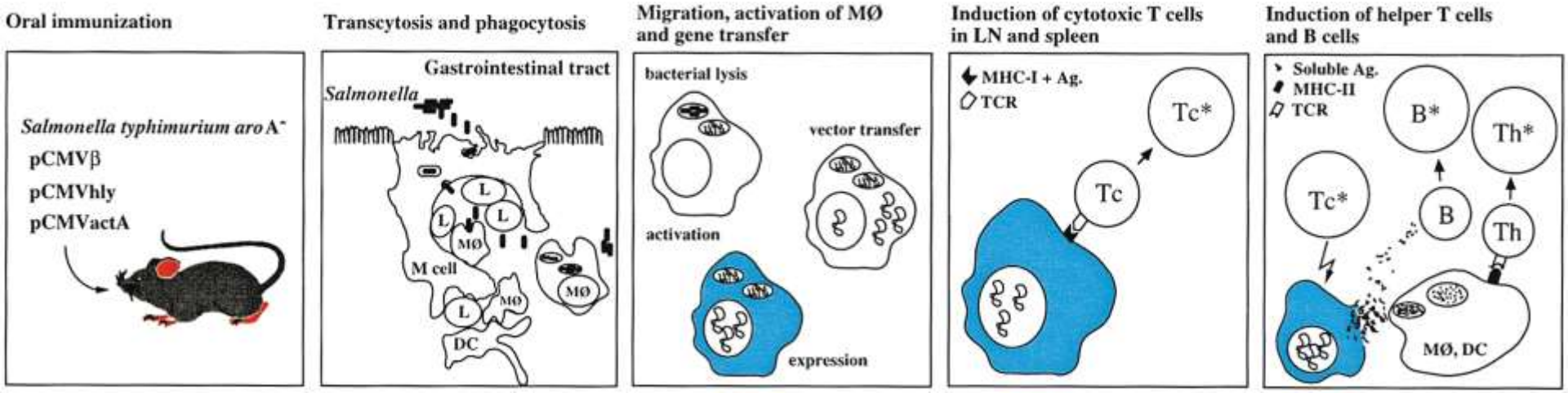
Genetic vaccination – biological DNA delivery



Genetic vaccination – biological DNA delivery
Oral somatic DNA vaccination by Salmonella ofr Shigella

Attenuated bacteria as a DNA delivery vehicle for DNA-mediated immunization

Bacteria carrying plasmids invade epithelial cells and lyze, because of lack od diaminopimelate ot aromatic aminoacids… release plasmid DNA..



VACCINE TYPES

- **Conjugate** (polysaccharide linked to immunogenic proteins; *Strep., H. influenzae* type B)
- **Experimental** (DNA vaccination, T-cell receptor peptide vaccines – modulation of cytokine production, proteins targeting complement inhibition, dendritic cell vaccines, recombinant vector introduction, reverse vaccinology…)
- **Valence** (monovalent – against single antigen or microorganism, polyvalent)

DIFFERENT MODEL SYSTEMS FOR INFECTIONS and vaccine testing

Animal models – widely used (ID_{50}, LD_{50}, adhesion, intracellular survival)
– legislatively more accessible
– for many purpose sufficient enough
– transgenic and „humanised“ animals

× disadvantages:

- differences in: immune system, microflora, receptors, sensitivity to pathogen…)
- non-natural ways of causing the infection: intraperitoneal, intracerebral (avoiding the colonisation step – „the first contact“)
- often more septicaemia than infection

Tissue cultures (monolayers, multilayers; dirrefentiated, polarized; primary, transformed - LIMITATIONS !)

Organ cultures

VACCINE DESIGN Considerations

- Response required
- Delivery pattern
- Antigen vs. Target disease
- Administration route
- Possibility of combination with other antigens
- Administration frequency

ADJUVANTS (Ramon, 1926)

- Immune system response enhancement
- Dose reduction
- Lowering the frequency of administration

Chemical substances:

- Oil emulsions:Freund's emulsified oil adjuvants (complete and incomplete)
  Arlacel A, Mineral oil, Emulsified peanut oil adjuvant (adjuvant 65)
- Mineral compounds
- Bacterial products (*Bordetella pertussis, Corynebacterium granulosum*derived P40 component, lipopolysaccharide, *Mycobacterium* and its components, Cholera toxin..)
- Liposomes
- Immunostimulating complexes (ISCOMs)
- Other adjuvants (Squalene)

PROTECTIVE IMMUNE RESPONSES

Immune response	**Mechanism**	**Localisation**	**Target antigens**
HUMORAL (antibodies)	Prevention	Blood Mucosa	Surface antigens
CELLULAR (T-helper 2)	Help antibody production	Lymph nodes	Any
CELLULAR (T-helper 1)	Local inflammation Help cytotoxic cells	Lymph nodes	Any
Cytotoxic T cells	Infected cells lysis	Lymph nodes	Any intracellular

PARAMETERS OF VACCINES FOR COMMERCIAL USE

According to the CPMP (Committee for Proprietary Medicinal Products):

- Geometric mean titer increase
- Seroconversion
- Seroprotection rate

Clinical trial phases:

- Phase I (20-30 subjects)
- Phase II (100 or more subjects)
- Phase III (hundreds to thousands subjects, usually randomized)

+ single-blind study vs. double-blind study
+ the FDA and the Office of Human Research Protections require that patients give **informed consent** before joining a clinical trial
+ vaccine patent application (composition, preparation, storage)

VACCINE SAFETY

- **Live attenuated vaccines**
  - insufficient attenuation
  - reversion to wild type
  - administration to immunocompromised patient
  - persistent infection
  - contamination
  - foetal demage
- **Non-living vaccines**
  - contamination (live organisms, toxins)
  - allergic reactions
  - autoimmunity
- **Genetically engineered vaccines**
  - oncogenes?

VACCINE DESIGN

Pathogenesis

Immune mechanism

Vaccine

	„Defense“	Antibodies	Th2	Th1	CTL
Live attenuated	Vaccinia Polio, BCG				
Whole cell killed	Cholera Polio, Pertussis	HAV			
Subunit		Diphteria Tetanus			
Purified subunit		Acellular Pertussis	Hib		
Recombinant subunit		Hepatitis B			
Adjuvanted subunit		Alum MPL MF59	MF59	Alum MPL SB AS-1	SB AS-1 SB AS-2
Live vectored				NYVAC Listeria Adenovirus	NYVAC Listeria Adenovirus
Nucleid Acid vaccination				+	+

MUCOSAL VACCINES

- **Anti-Infectious**
  - enteric infections
  - other mucosal infections
- **Anti-Inflammatory**

- certain infections
- autoimmune diseases
- allergies
- transplantations

Approaches to mucosal immunisation

- Oral, intranasal, intrarectal, intravaginal
- Stimulation of local and systemic responses

Living microbes: Salmonella,Shigella
BCG
Adenovirus, poxvirus

Non-living antigens: Microencapsulated Ag
Killed microorganisms
Adhesins from bacteria
Transgenic plants

REGISTRED VACCINES
(since 2000)

Hexavalent combination for infants

- since 1.1. 2009: INFANRIX hexa™
- Diphtheria, Tetanus, Pertussis, Hepatitis B, Polio, and *Haemophilus influenzae* type b
- for the routine infant primary immunization series at 2, 4 and 6 months of age

Rotavirus (Oral)

- RotaTeq™, Rotarix™

Pneumococcal Conjugates
- Prevenar™ (heptavalent, multiple serotypes)

Meningococcal Conjugates
- MENVEO™ (serotypes A, C, Y and W135)

MMR-Varicella
- measles, mumps, rubella/+ varicella

Current PRINCIPAL VACCINE TARGETS

Chlamydia pneumonia

CMV

Human papilloma

Malaria

EBV

Tuberculosis

HIV

Meningococcus B

Group B Streptococci

…

Mass Vaccination Reduced by >97% the Incidence of 9 Devastating Diseases and Eliminated 2 of Them (Smallpox and Poliomyelitis)

Disease	Max. N° of cases (year)	N° of cases in 1997	Reduction
Smallpox	48,164 (1901-1904)	0	**100%**
Poliomyelitis	21,269 (1952)	0	**100%**
Diphtheria	206939 (1921)	5	**99.99%**
Measles - spalničky	894134 (1941)	135	**99.99%**
Rubella - zarděnky	57686 (1969)	161	**99.72%**
Mumps - příušnice	152209 (1968)	612	**99.59%**
Pertussis	265269 (1934)	5519	**97.92%**
H. influenzae	20000(1992)	242	**98.79%**
Tetanus	1560 (1923)	43	**97.24%**

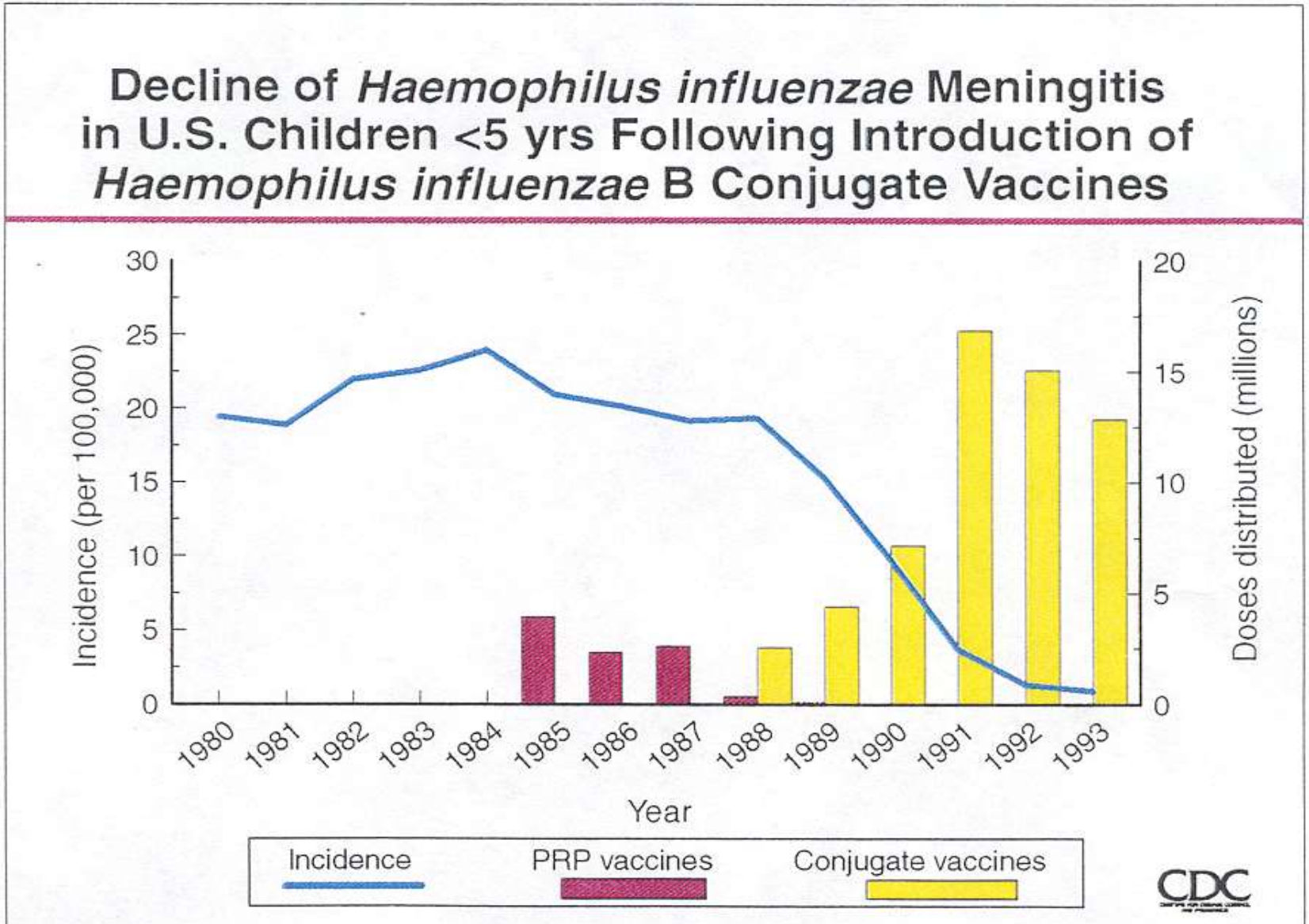
One of the goals that can be achieved within the next decade is the elimination of

BACTERIAL MENINGITIS

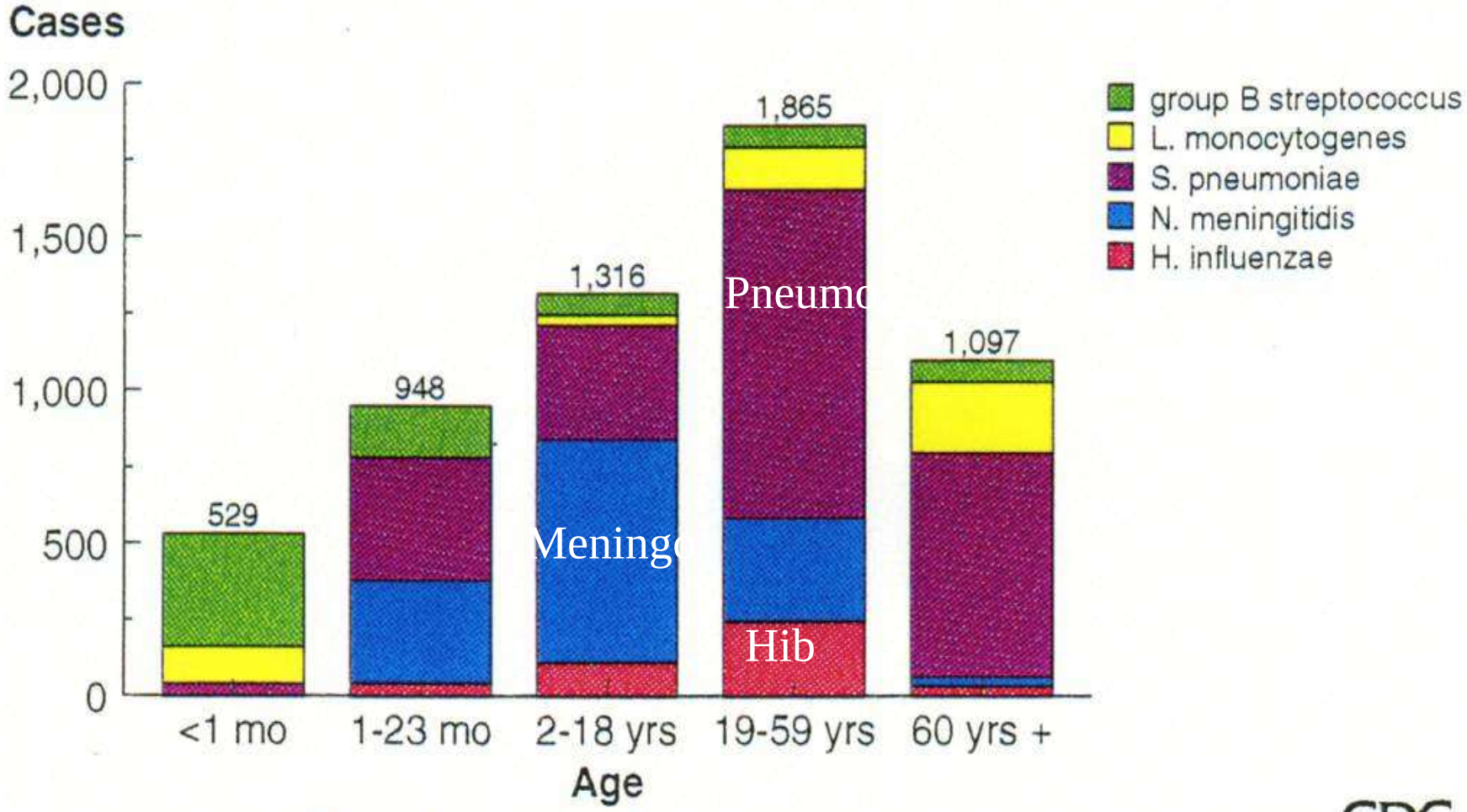
1980's	USA	UK	S. Africa
Haemophilus influenzae	48%	29%	36%
Neisseria Meningitidis	20%	25%	50%
Streptococcus Pneumoniae	13%	20%	12%

180,000 deaths / year

The Hib vaccination has eliminated the disease caused by Haemophilus influenzae from all those countries which adopted the vaccine



Cases of Bacterial Meningitis, U.S., 1995, by Pathogenic Agent and Age Group



*Schuchat et al, NEJM 1997, Vol. 337, No. 14

CDC

MENINGOCOCCAL MENINGITIS

350,000 cases / year
35,000 deaths

MORTALITY	10 - 15 % (within 24-48 hours)
SEQUELAE	7 - 25 %
ENDEMIC CASES	~ 1 / 100,000

1995	USA	2,800	cases
1997	EU	5,600	cases

Serogroups A, B, C, Y, W135

EPIDEMICS

New Zealand	17 / 100,000	(group B)
Sub Saharan Africa	>100 / 100,000	(group A)

The Legacy of Bacterial Meningitis in infancy

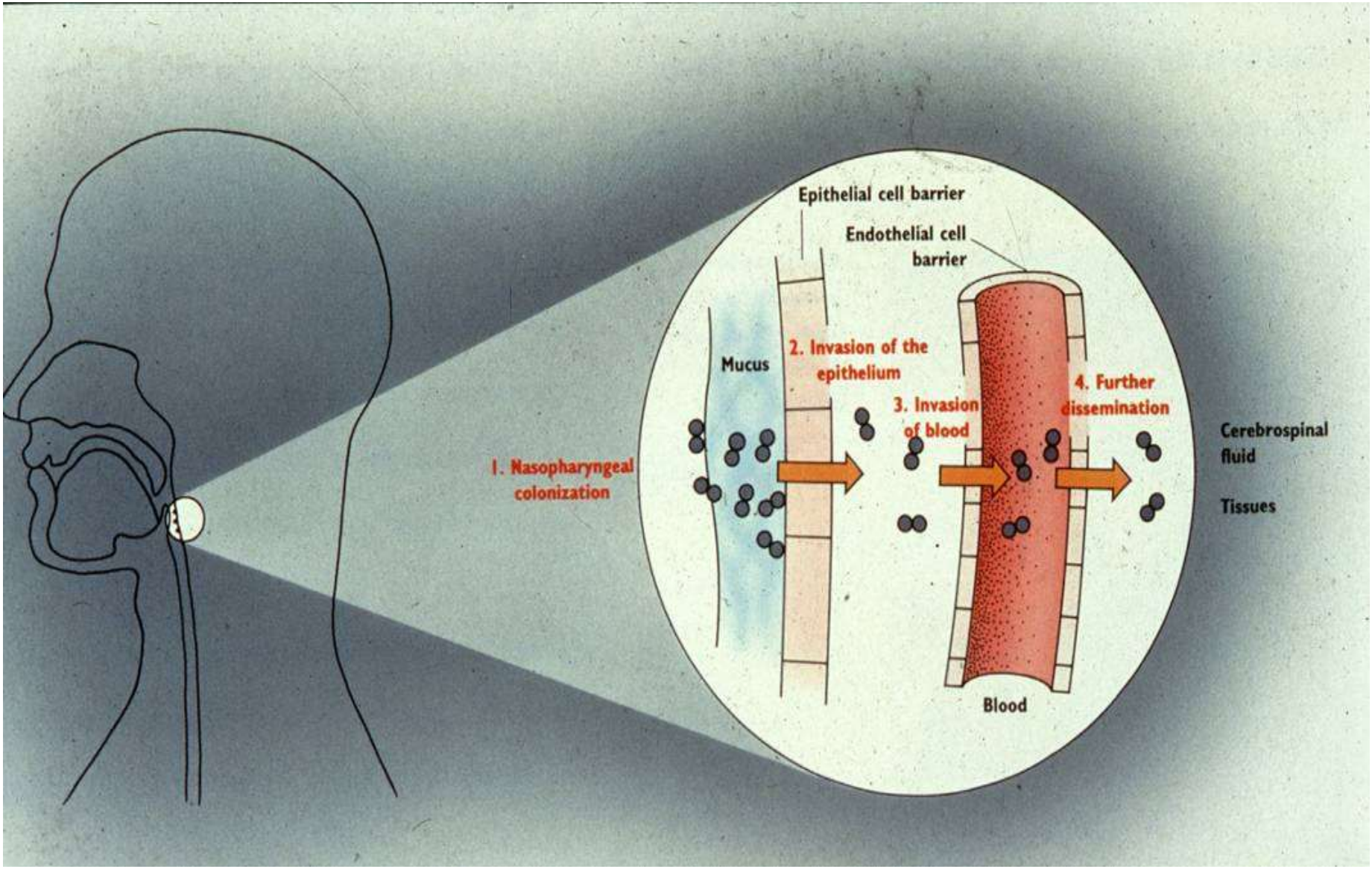
BMJ 323 1-5, September 2001

- Study on 1717 children surviving acute attack of bacterial meningits, compared to a similar number of controls (numbers below are % incidence over controls by the age of 5)
- 1.8% died within 5 years
- 20% had severe or moderately severe disability
- Learning difficulties 6.5%
- Neuromotor disabilities 7.2%
- Seizure disorders 4.6%
- Hearing problems 12.1%
- Ocular or visual disorders 9.8%
- Speach or language problems 11.1%
- Behavioural problems 8.6%
- Subtle deficits were also prevalent

Bacterial meningitis can be eliminated within 2010

Pathogen	*Vaccine Status*
Hib	Available
Pneumococcus	Available
MenC	
MenY, W, A	
MenB	????? The last obstacle…..

Commensal vs. virulent behavior



Obtained from R. Moxon

Vaccines against N. meningitidis

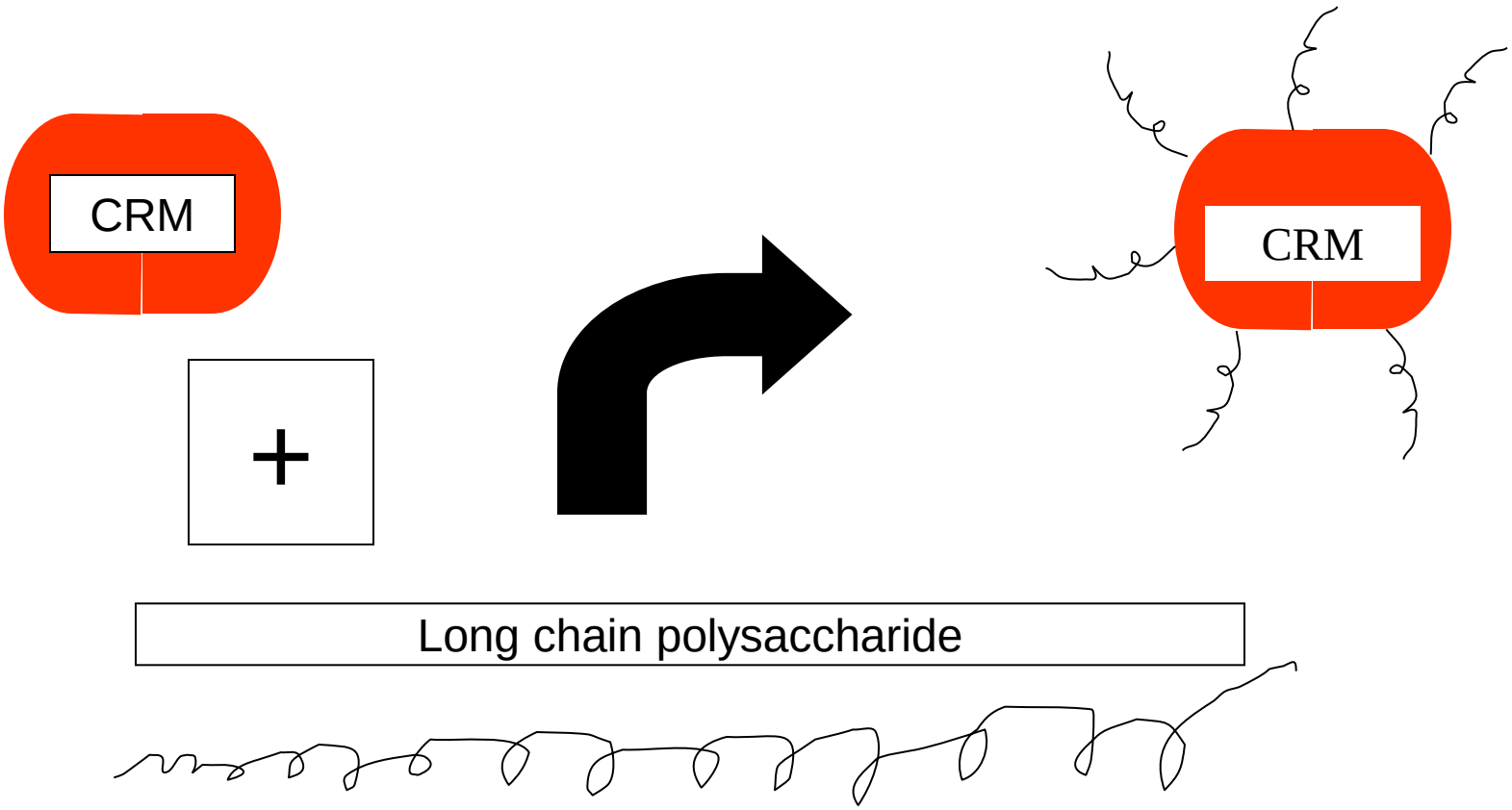
- Capsular polysaccharide against MenA, C, Y, W135

  developed in the 1960's

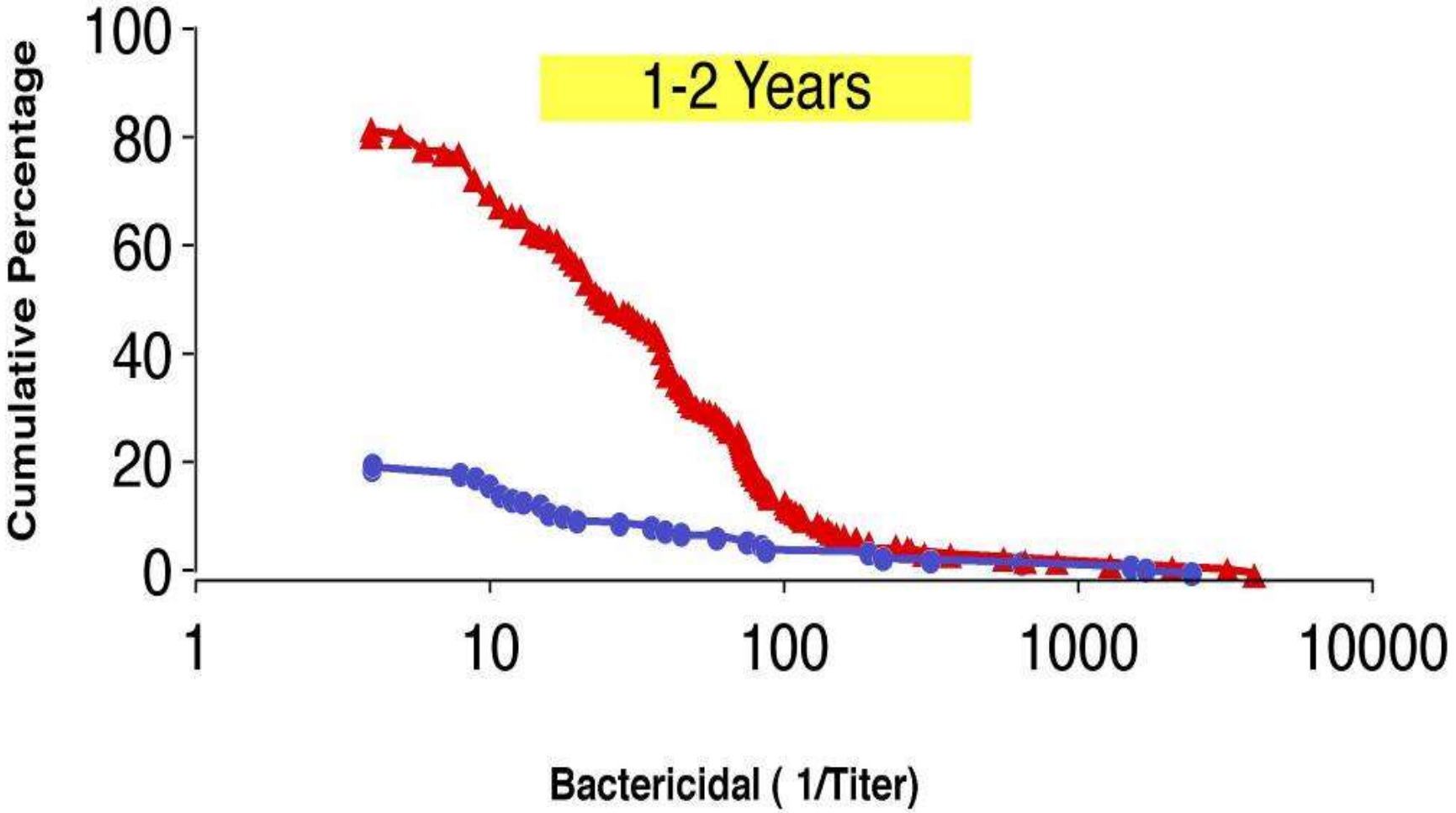
  They work well in adults but induce only a primary response, no memory, they do not work in infants (below 2-5 years)

- No vaccine against B

Ploysaccharides and conjugates



MenC conjugate vaccine (---) induces high level of bactericidal antibodies in infants. Plain polysaccharide (---) is a poor immunogen

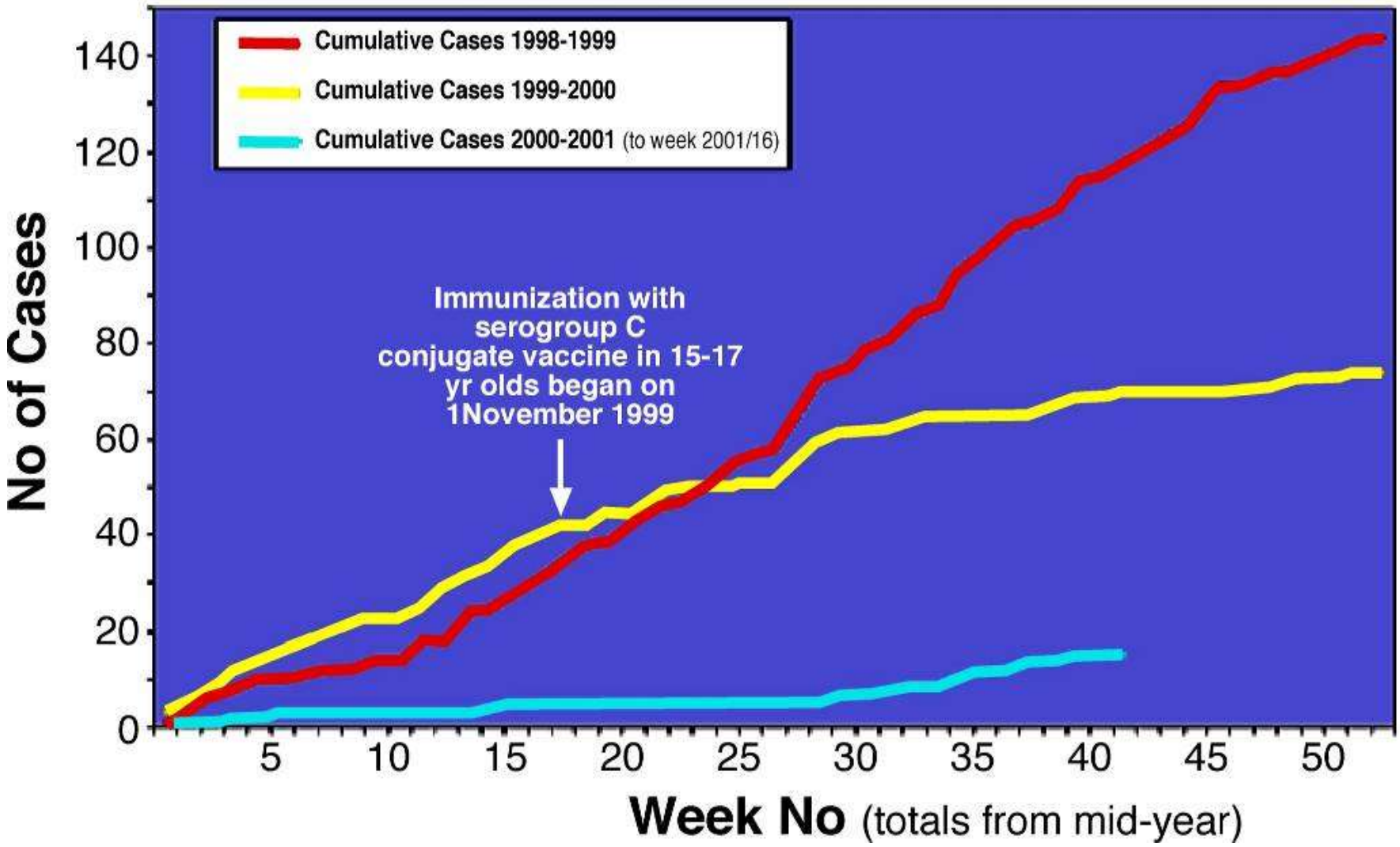


The MenC conjugate vaccine is efficacious

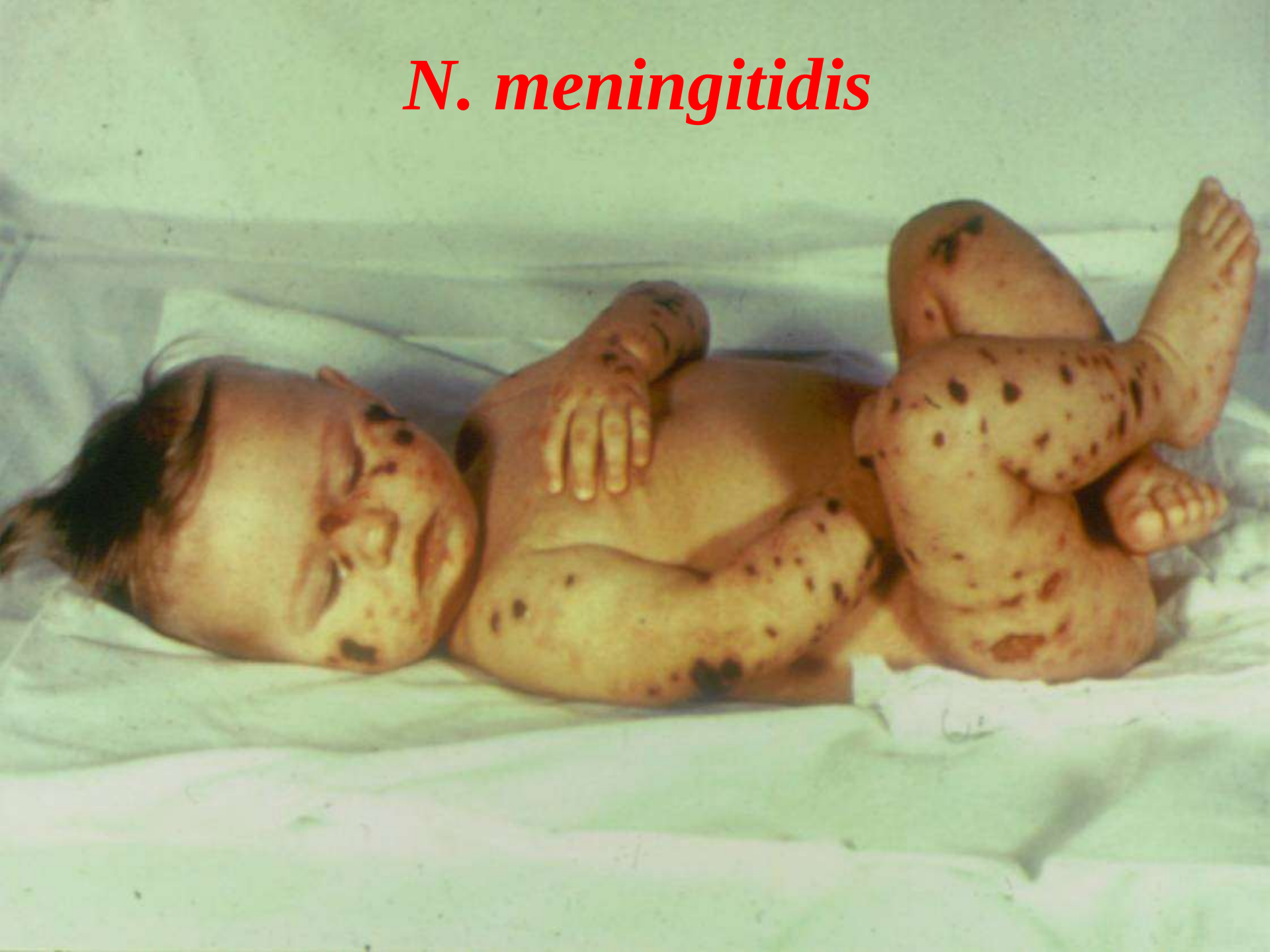
- The efficacy of the MenC conjugate vaccine in the UK has been:
- 97% in adolescents
- 92% in infants

- Vaccination of 2mo-18 years olds prevented
- >1500 cases
- >200 deaths
- > sequelae??

Laboratory Confirmed Cases of Serogroup C Meningococcal Disease (England & Wales)



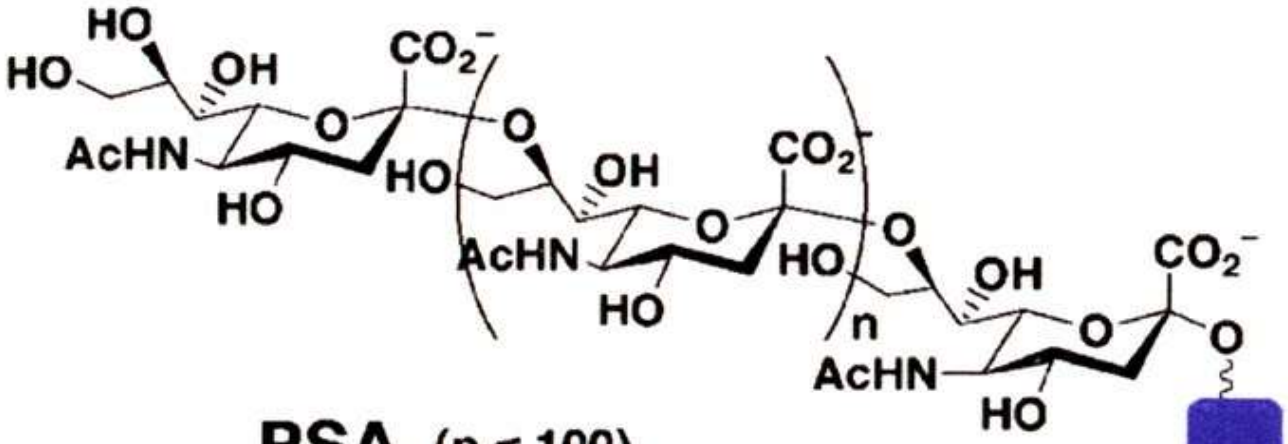
N. meningitidis



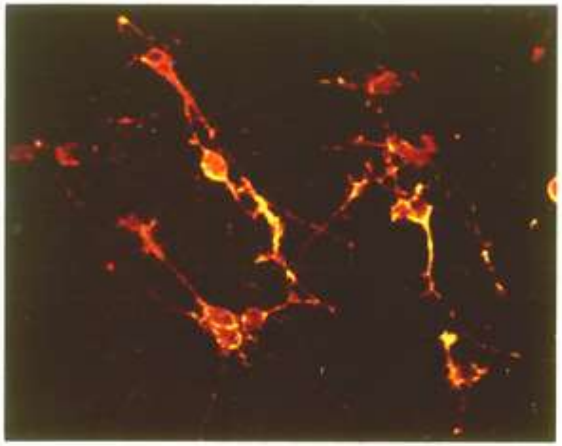
Bacterial meningitis can be eliminated within 2010

Pathogen	*Vaccine Status*
Hib	Available
Pneumococcus	Available
MenC	Available
MenY, W, A	Development/no technical problems foreseen
MenB	????? The last obstacle…..

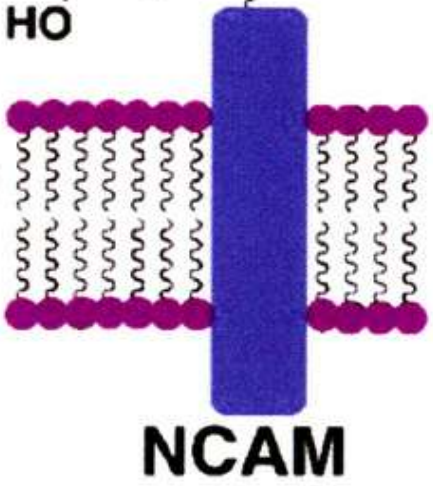
MenB capsular polysaccharide is a self antigen



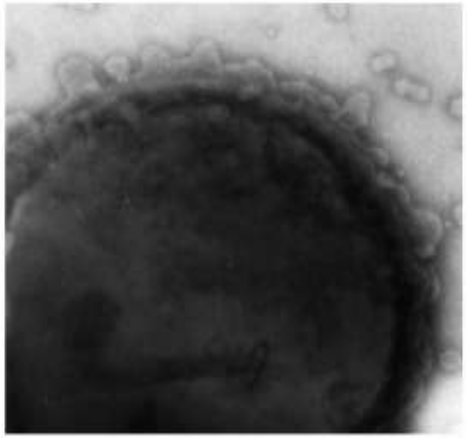
PSA ($n \leq 100$)



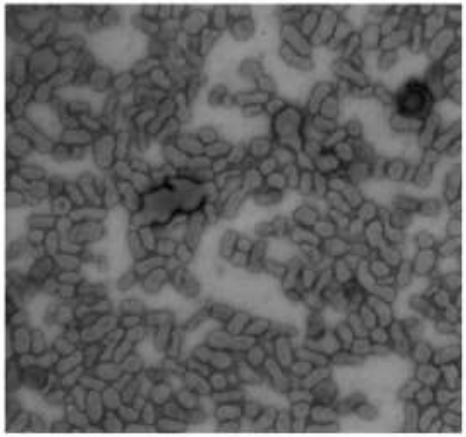
Neuron or tumor cell membrane



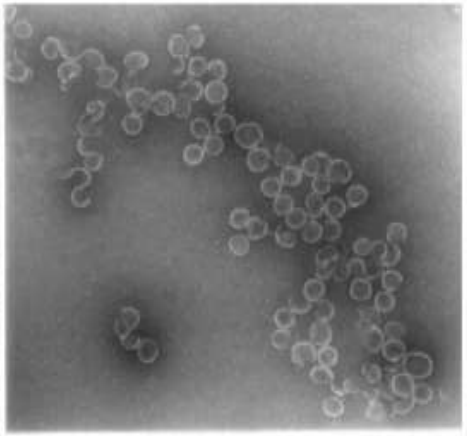
Electronmicrographs of meningococcal vesicles



"blebbing" meningococcus



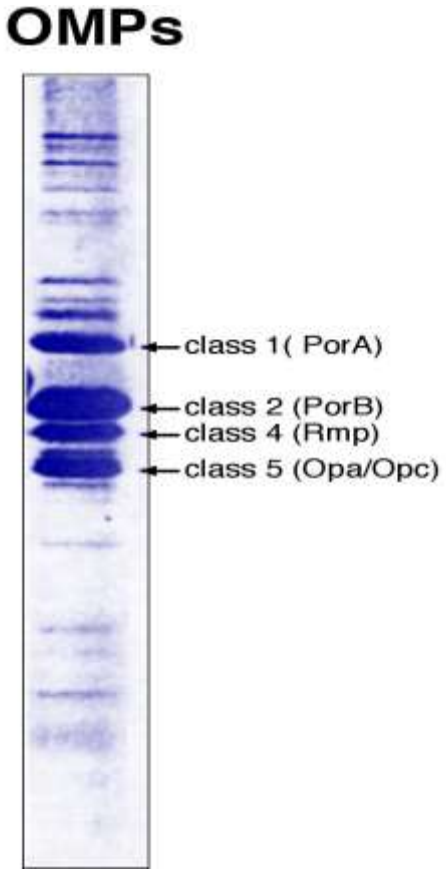
extracted vesicles (intermediate products)



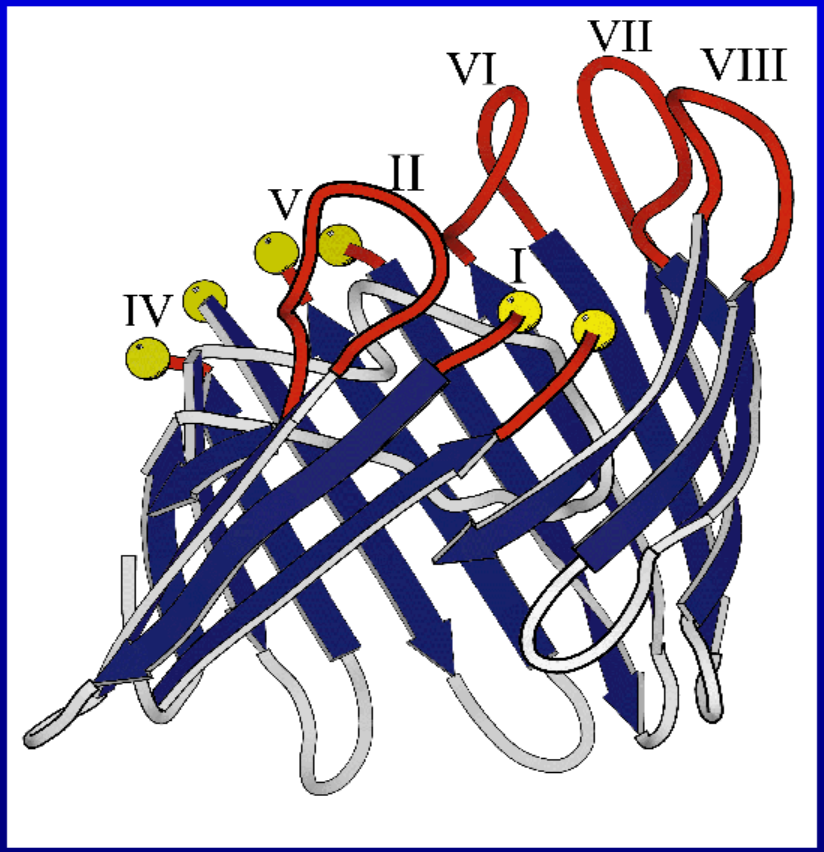
purified, LPS-depleted vesicles (bulk product)

Cuban/Norwegian OMV Vaccines
(The only MenB vaccines with efficacy data)

- Outer Membrane Vesicles (OMV) are obtained by detergent extraction of whole bacteria.
- The vaccine contains small membrane vesicles containing the most abundant proteins of the outer membrane (PorA and PorB are the main components) and small amounts of deoxycholate and LPS (lipopolysaccharide) and some minor proteins.

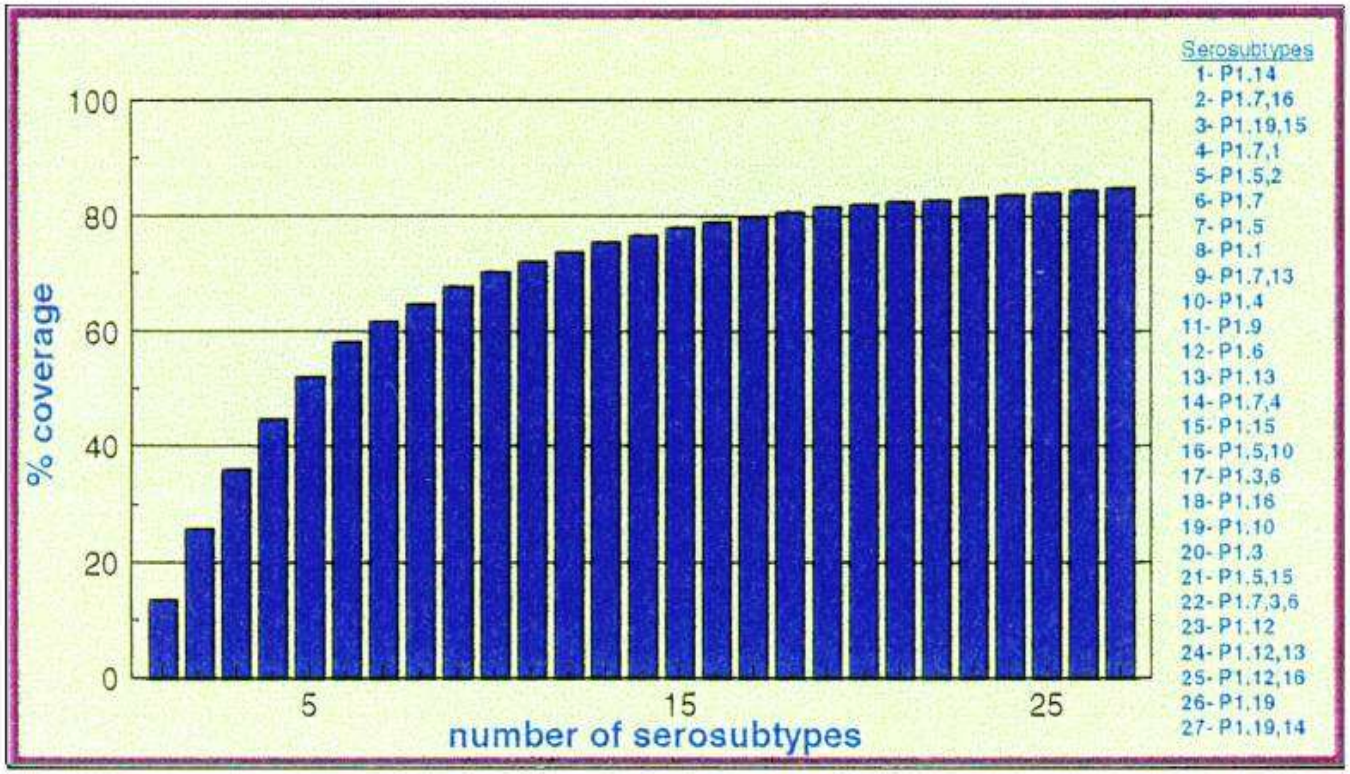


PorA monomer homology model

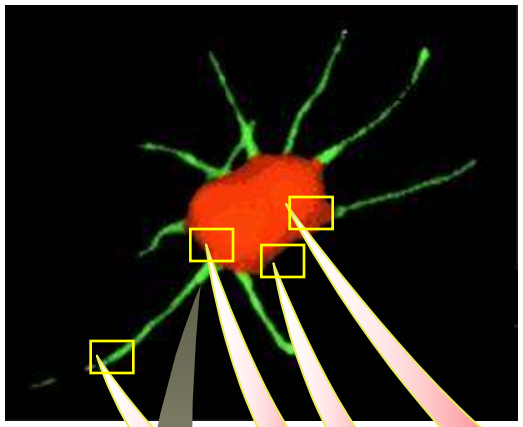


CDC/OMP

Number of serosubtypes that would have to be added in a multivalent OMP-based vaccine versus percentage of *N. meningitidis* serogroup B sporadic disease coverage in the U.S., 1992-1998



Conventional vaccine development failed to provide an universal vaccine



Cultivate Microorganism

5-15 years

Antigen selection

Clone genes

Test Convalescent sera

Test immunogenicity

Purify components

Identify components

Immunogenicity testing in animal models

VACCINE DEVELOPMENT

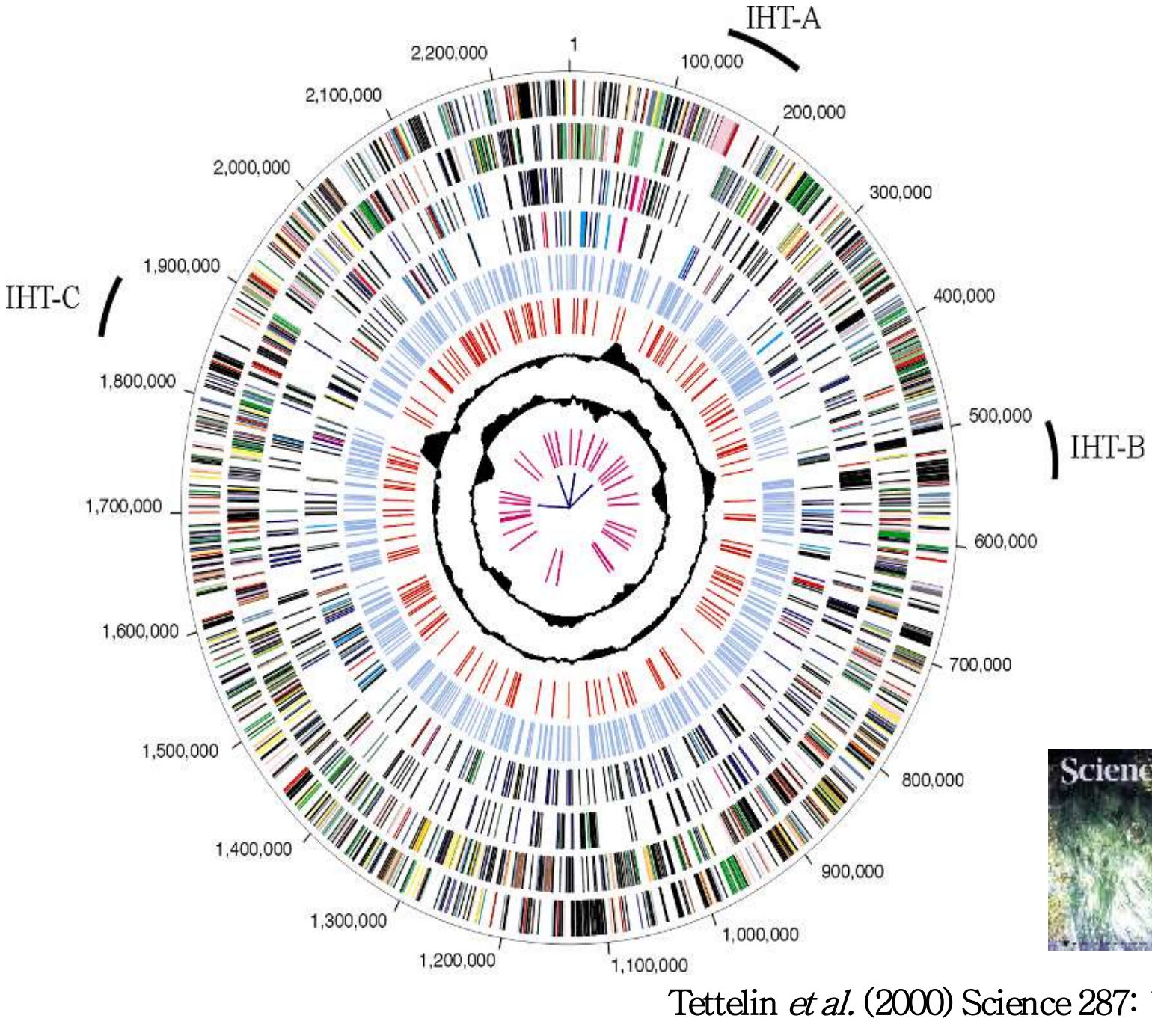
Vaccine

3 groups' synergy

The Institute for Genomic Research, USA

Chiron Corporation, Italy

Oxford University, UK



Tettelin *et al.* (2000) Science 287: 1809–1815

Reverse Vaccinology

Craig Venter and Flaire Fraiser

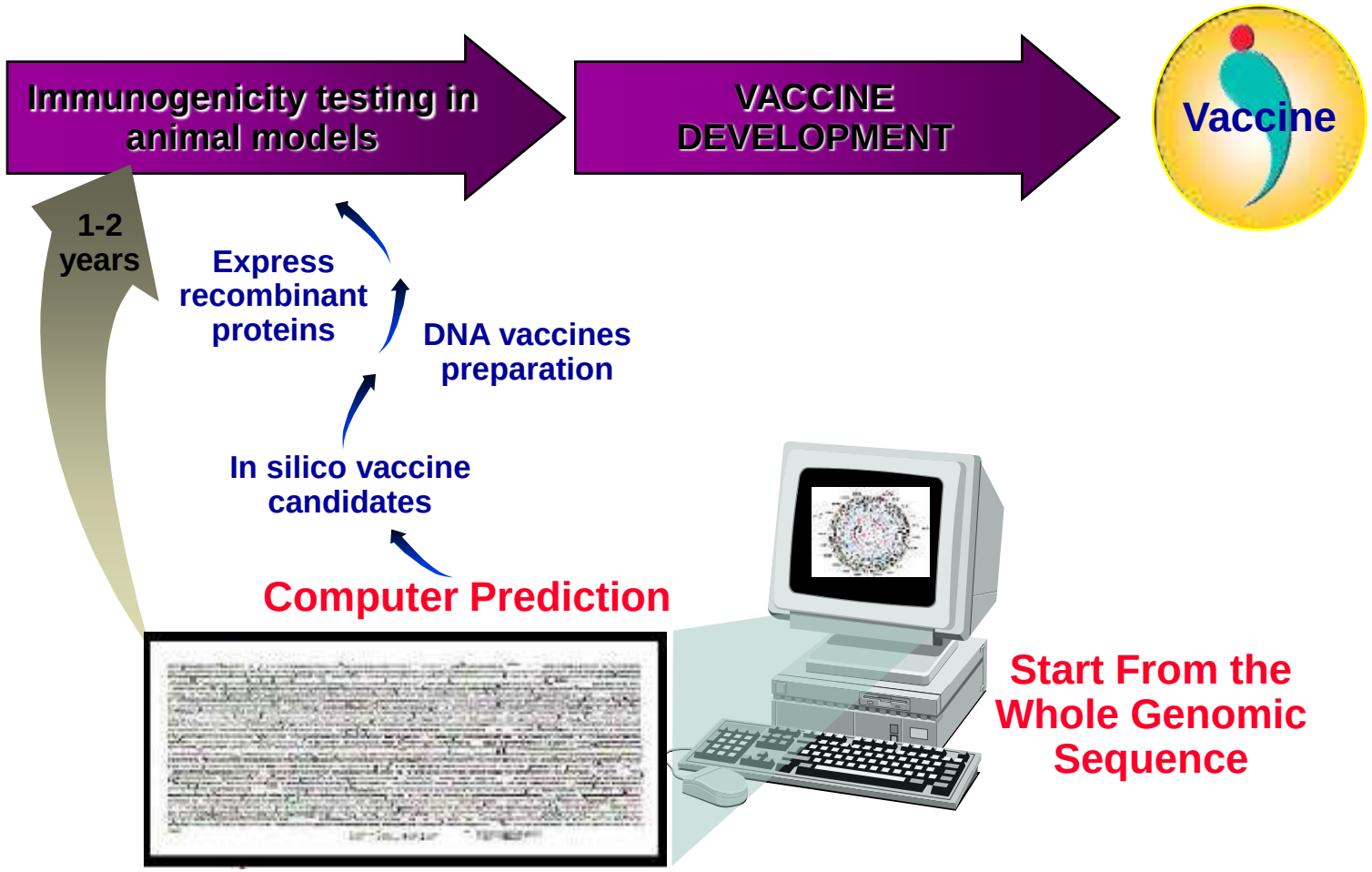
(May 11, 2001)

Completed genome of Group B *Neisseria meningitidis* (strain MC58)

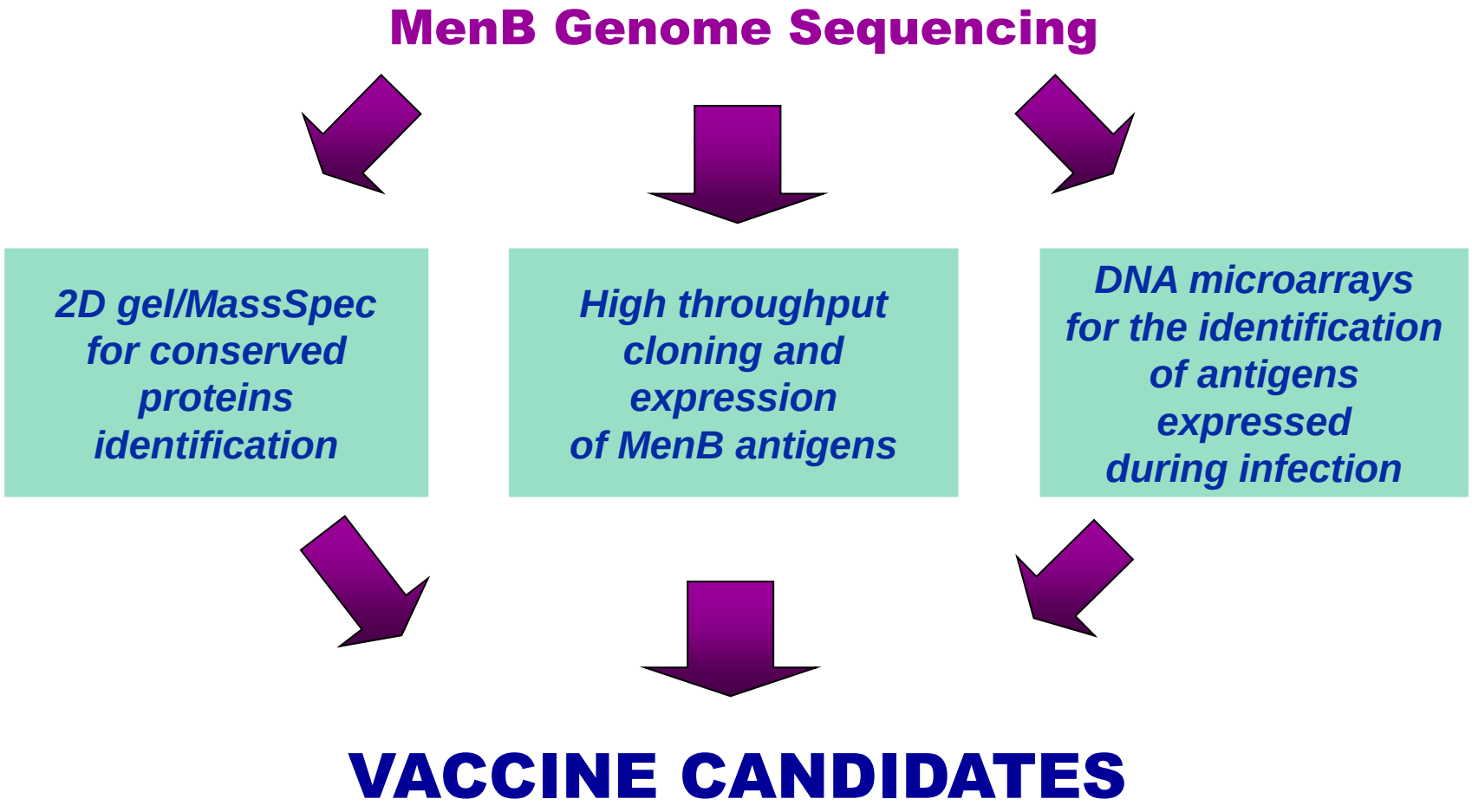
TIGR, Chiron Vaccines and Oxford University Research Consortium

MenB Vaccine: Genomic-Based Vaccine Development

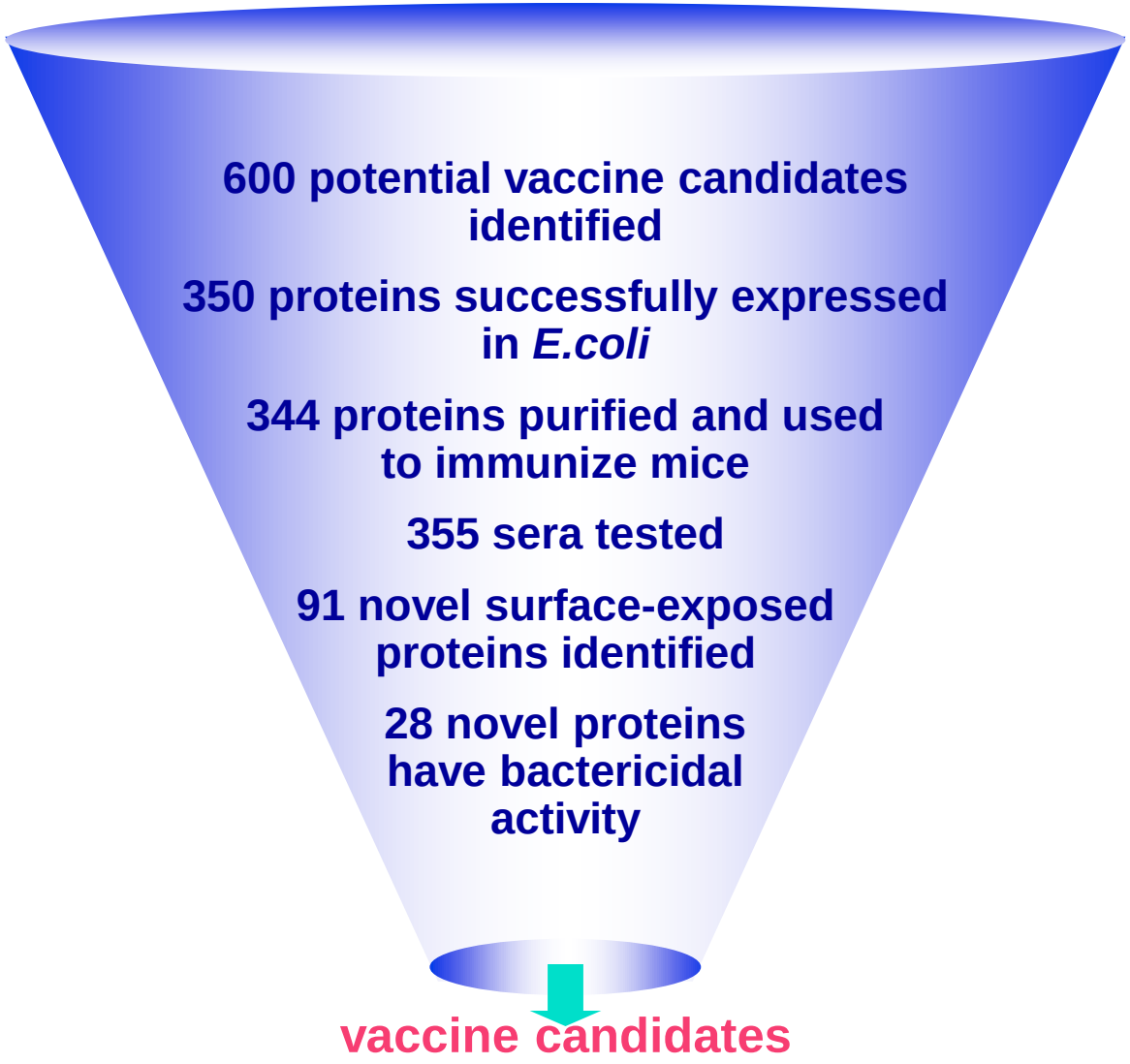
Reverse Vaccinology



MenB Vaccine: A Genomic Approach



MenB Vaccine: A Genomic Approach

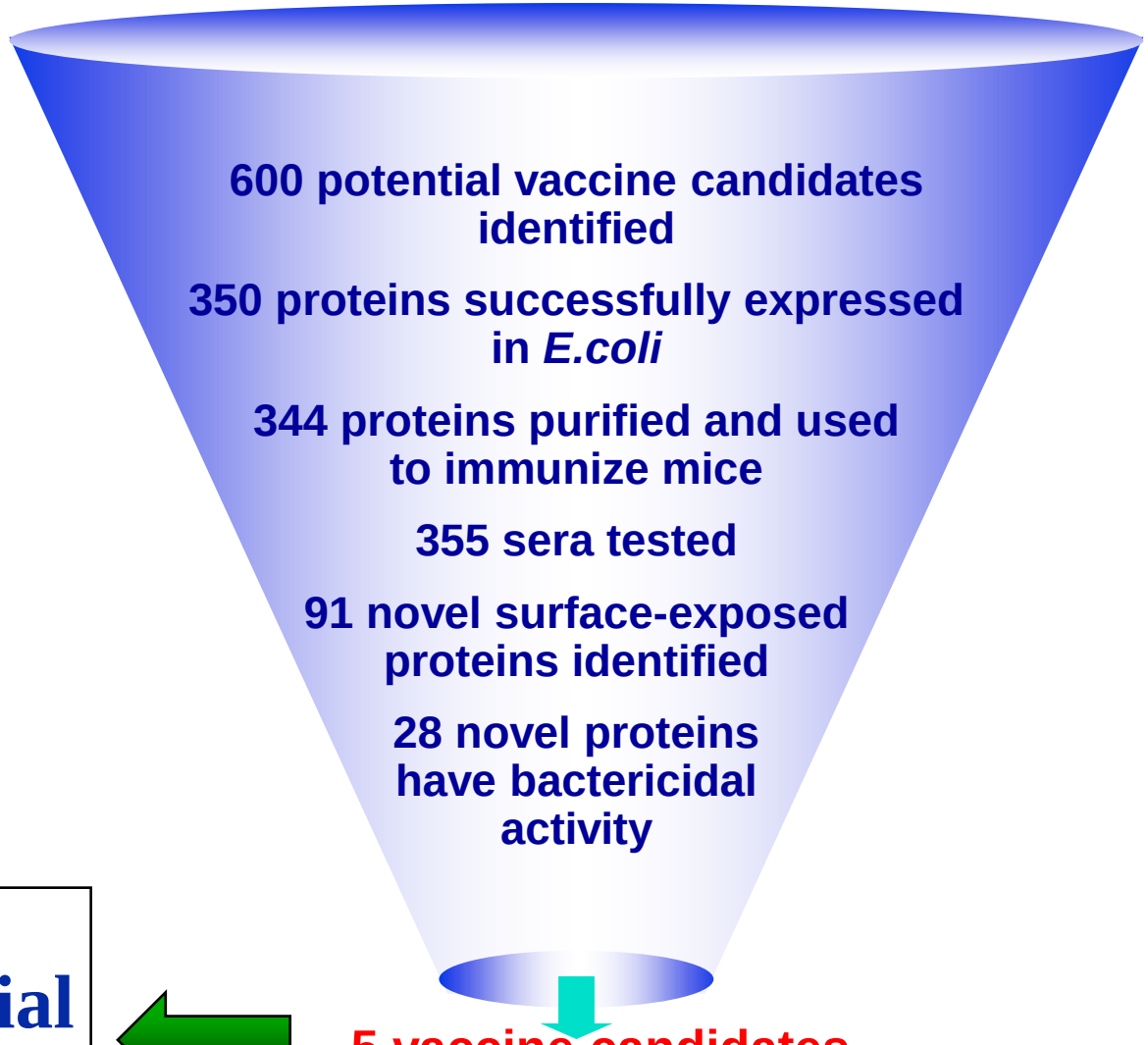


Properties of the New Proteins

Properties of the new proteins

Antigen	Remarks	FACS	ELISA	Bactericidal titer
GNA33	Lipoprotein	++++	13,000	1/16,000
GNA992	Outer membrane protein	+++	2,750	1/256
GNA1162	Lipoprotein	++	1,270	1/4
GNA1220	Membrane protein	+++	1,000	1/256
GNA1946	Lipoprotein	+++	13,100	1/32
GNA2001	Outer membrane protein	++	500	1/512
GNA2132	Lipoprotein	++	1,700	1/16,000
GST	-	-	< 50	< 1/4
OMV	Mixture of proteins containing mainly PorA	++++	260,000	1/32,000

MenB Vaccine: A Genomic Approach



18 months

24 months

Scale-up
GMP
IND
1 year

Clinical trial

Other pathogens that can be addressed by reverse vaccinolgy

- Malaria
- Tuberculosis
- Treponema
- Pneumococcus
- Group A streptococcus
- Group B streptococcus
- Staphylococcus
- Pseudomonas
- ……
- Any pathogen can be approached ……

Reverse vaccinology for viruses

- Due to their small size, viral genomes have been available for more than two decades
- however, the approach to vaccine development has been conventional: only structural (envelop and core) antigens have been usually considered for vaccine development
- Very recently the concept of reverse vaccinology has been applied also to viruses and all encoded proteins are being considered as potential antigens
- Promising results with HIV early proteins such as Tat, Rev, Pol, etc show that the approahch is a winner also in this case

Gene expression in *N. meningitidis* under iron starvation

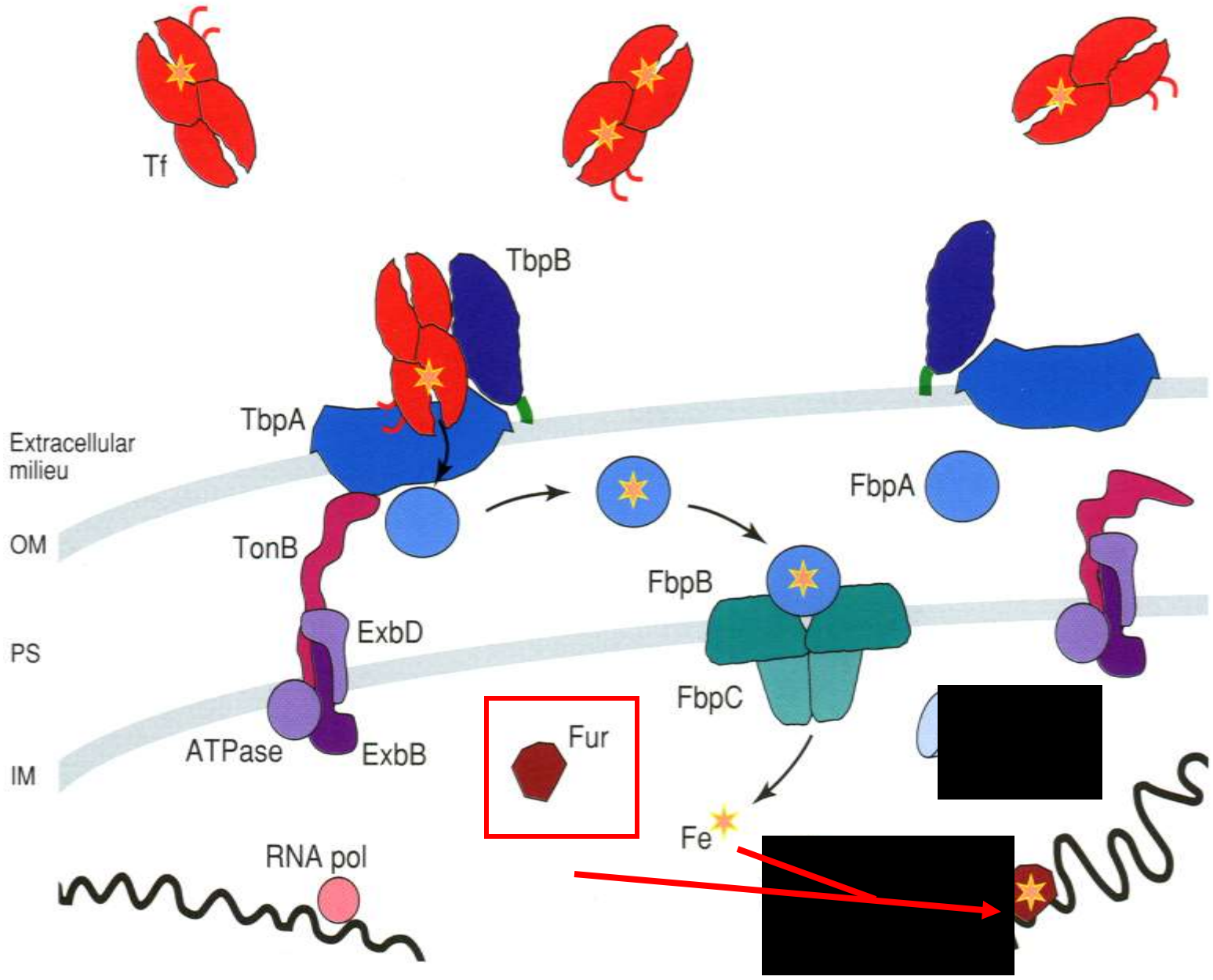
In human body more than 99,9% of iron is bound to transport (transferrin, lactoferrin) and storage proteins (ferritin, heme-containing compounds)

For invasion and proliferation bacteria need to induce specific pathways capable of scavenging iron from the host

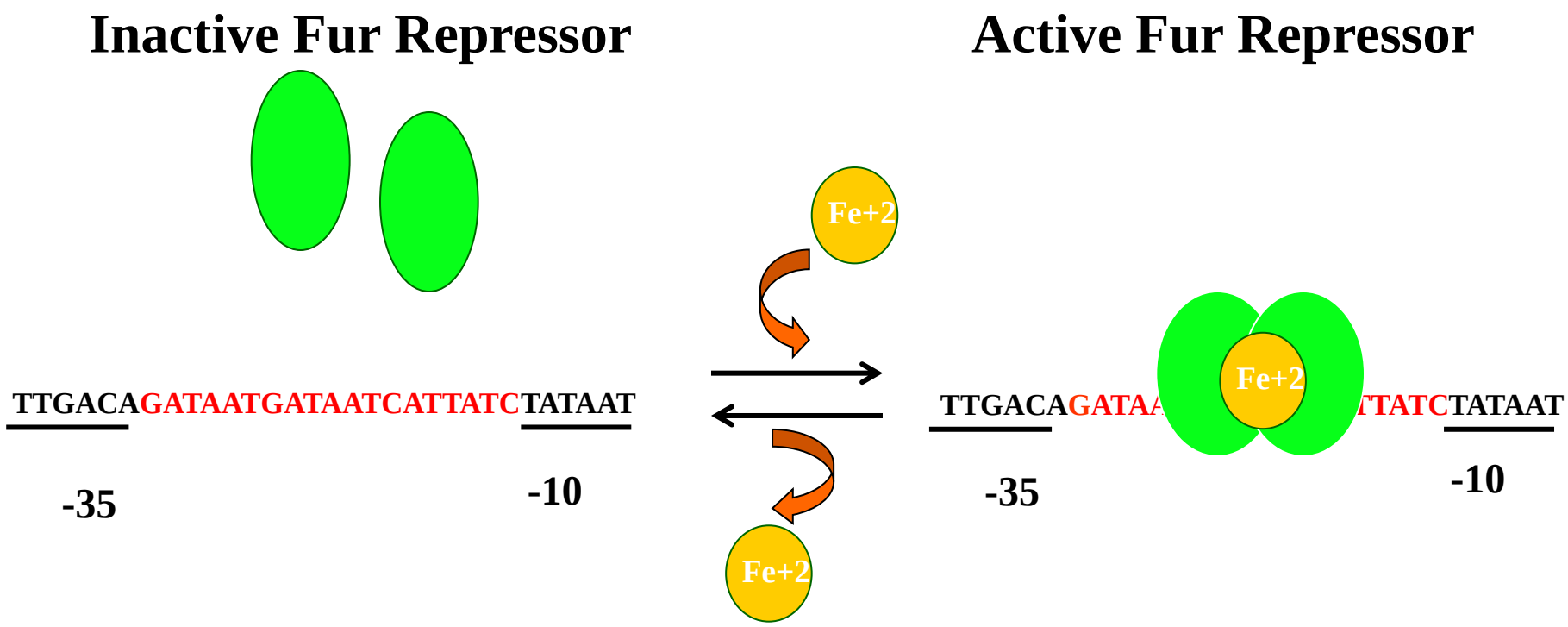
Several *Neisseria* virulence genes are iron-regulated

IRON ACQUISITION PATHWAYS IN PATHOGENIC *Neisseria*

Iron donor	Surface receptor
transferrin lactoferrin	TbpA, TbpB LbpA, LbpB
Haem, Haemoglobin (Hb) Hb-haptoglobin (Hp) Haem-Haemopexin Haem-albumin	HmbR and HpuA/B HpuA/B Not utilizable Not utilizable
Siderophores from other bacteria	FrpB (homologue to ferric enterochelin receptor fepA)

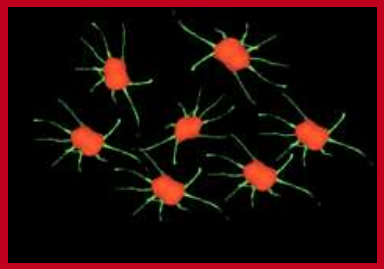

Tf
TbpB
TbpA
Extracellular milieu
OM
PS
IM
TonB
ExbD
ExbB
ATPase
FbpA
FbpB
FbpC
Fur
Fe
RNA pol

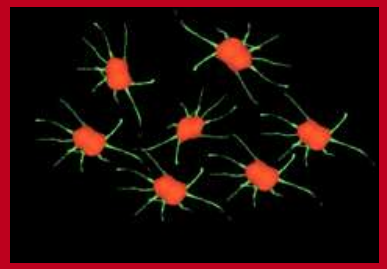
MECHANISM OF Fur REGULATION



+ $Fe(NO_3)_3$

+ Desferal



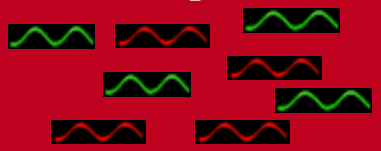


Cy5

Cy3

+

Probe



Target: PCR products

Chip

Hybridization

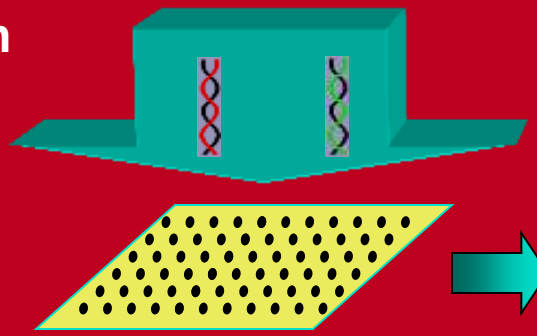
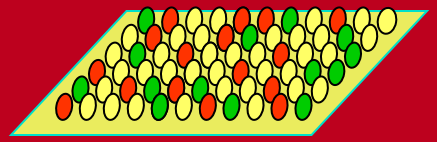
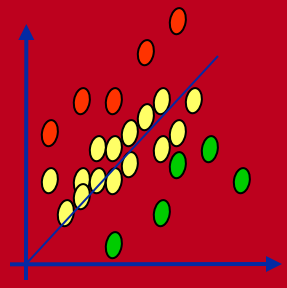


Image processing



Data mining and visualization



GENES AFFECTED BY IRON STARVATION

UP-REGUTATED GENES

- Iron acquisition and transport mainly via transferrin and lactoferrin pathway
- Pilus assembly and modification
- Outer membrane proteins
- Oxidative stress response and other stress responses
- Energy metabolism genes (glycolysis, TCA cycle)
- Transcriptional regulators
- Hypothetical genes

DOWN-REGULATED GENES

- Cell wall biosynthesis
- Protein synthesis machinery
- Outer membrane proteins
- AA transport
- Iron storage
- Electron transport
- RNA processing

frpA	GATAATTAATTTATT
tbpA	ATAATGATAATCATTAT
fur	GATAATCATACGCTTAAGC
fbp	GATAATAACAAA-TTTAAAA
tbpB	AATAAATAAAATAATAATC
iroA	AATAATGATGGGAAA--TC-TC
lbpA	GATATTGAAAATGAAGTTG
hmbR	TTAAATATTAATGATTATCA
frpB	AAAAATAATTATTATTATTTTTT
Neisseria consensus	GATAAT-ATAATAATTATC-TTT
Escherichia coli consensus	GATAATGATAATCATTATC

263 regulated genes

↓

211 transcriptional units

↓

77 Fur boxes (13/21 matches)

Experimental confirmation (EMSA)

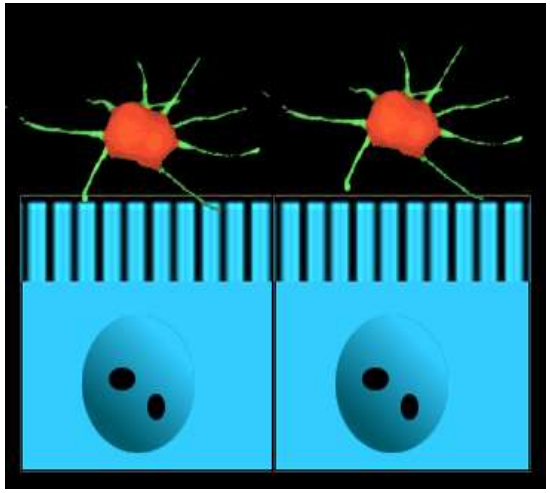
Conclusions 1

- 263 Iron-regulated genes were revealed by microarray analysis
- New iron-dependent genes regulated by Fur have been identified
- (including hypothetical genes)
- Fur acts as positive and negative regulator in *N. meningitidis*
- Gene expression profiling is a good predictor of Fur-regulated genes
- 51% of genes up-regulated during iron starvation are predicted as
- surface located, potential vaccine targets

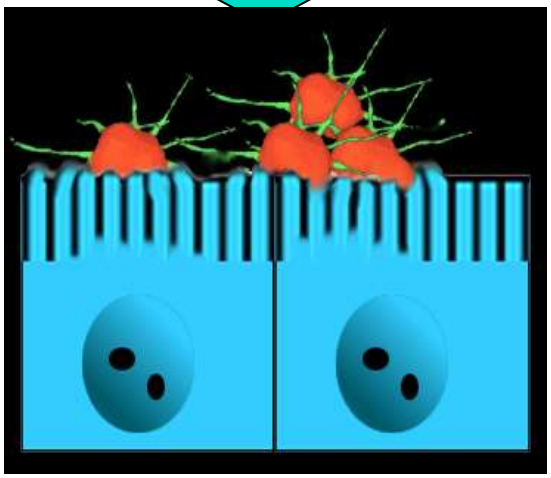
1. **BACTERIA GROWN UNDER IRON STARVATION**

2. **BACTERIA ADHERING TO EPITHELIAL CELLS**

Interaction of *N. meningitidis* with an epithelial monolayer



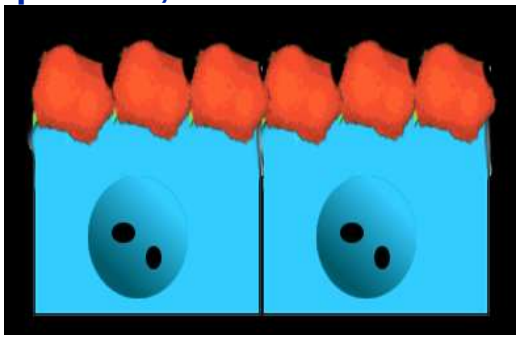
PilC + bundle forming pili



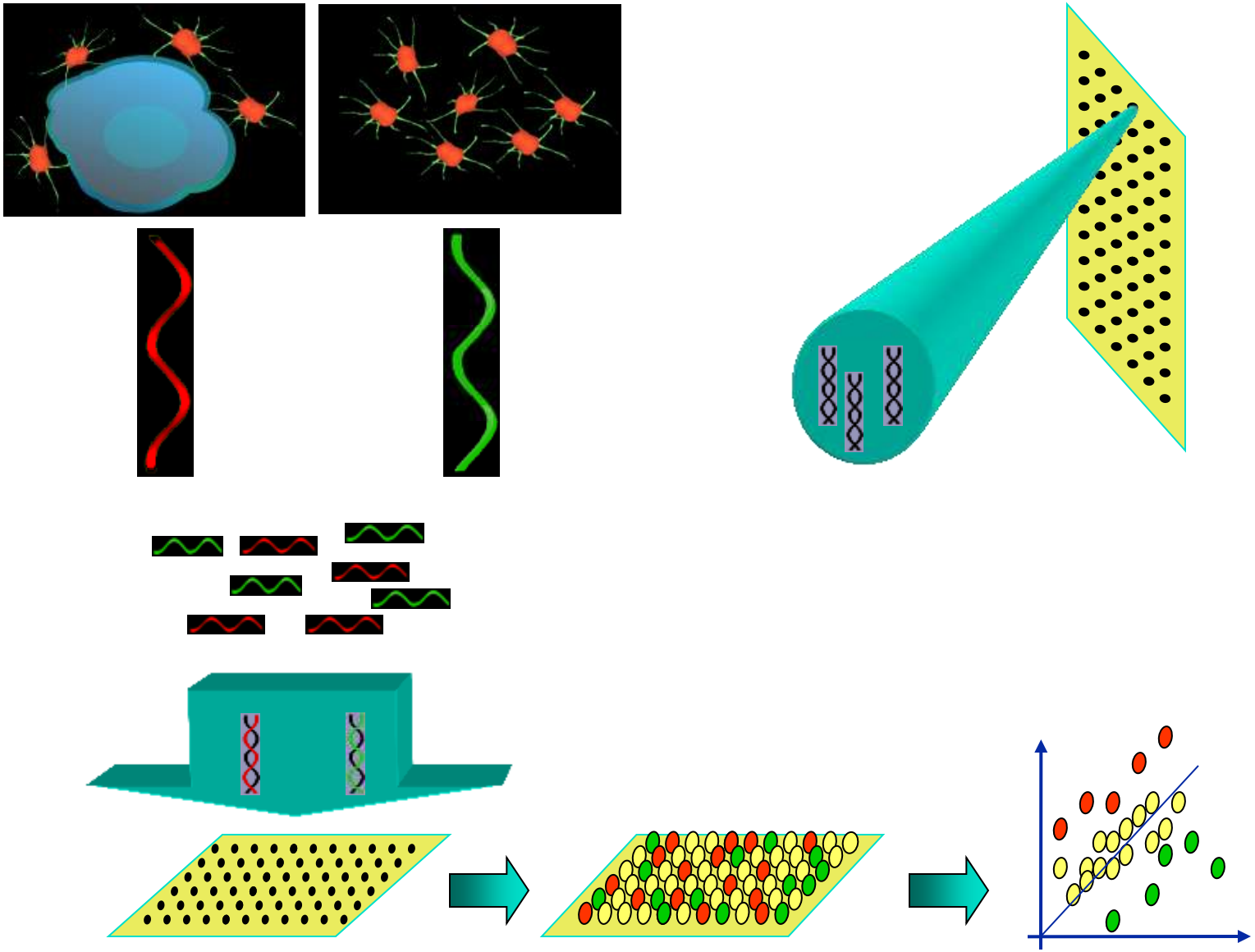
Localized adherence

PilT

Diffuse adherence (loss of piliation, intimate attachment)



Microarray identification of genes induced by meningococcal contact with epithelial cells



Regulated gene families	Epithelial cells 16HBE14			Epithelial cells CHANG			Endothelial cell HUVEC		
	↑	↓	↕	↑	↓	↕	↑	↓	↕
Unknown proteins	35	54	9	28	2	nd	56	87	11
Iron acquisition, storage and mobilization	12	2	0	5	1	nd	8	3	0
Transporter (AA, sulphate, unknown solute)	14	8	0	6	6	nd	18	7	4
Sulphur methabolism	4	0	0	4	0	nd	4	0	0
Pathogenesis (pilus and capsule regulation, adhesins)	1	5	2	1	2	nd	4	15	2
Toxin production and resistance	3	1	1	3	0	nd	1	2	0
Transcriptional regulations	4	0	0	1	0	nd	3	0	0
Detoxification proteins	0	1	0	1	0	nd	0	0	1
Energy methabolism	4	9	0	2	8	nd	20	14	3
Protein synthesis	9	10	3	2	3	nd	3	44	6
Transposases	1	8	1	2	0	nd	0	19	1

SUMMARY OF DNA MICROARRAY ANALYSIS

347 GENES ALTERED THEIR TRANSCRIPTION LEVEL ONCE

-GENES ESSENTIAL FOR CELL DUPLICATION (down-regulation of genes involved in protein and nucleotide synthesis, cell wall septation and synthesis, ATP synthase F1 and F0 subunits)

-ADHESION GENES

- HOST-PATHOGEN CROSS-TALK GENES (up-regulation of transport machineries including iron, chloride, amino acids, ammonium and sulfate)

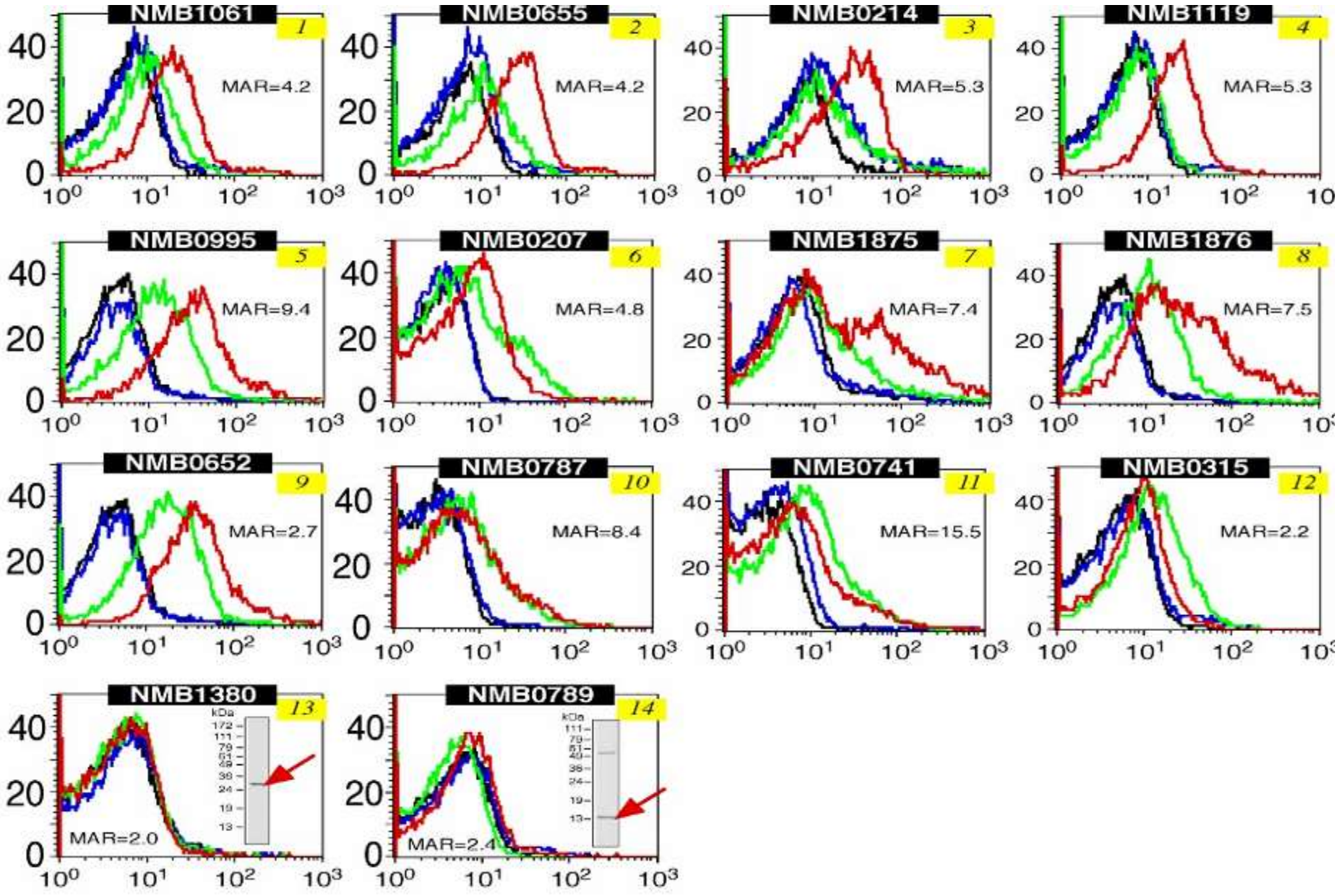
-AMINO ACIDS AND SELENOCYSTEINE BIOSYTHESIS

-

-DNA METABOLISM GENES (methylases, nucleases, transposases, ligases, helicases)

- OTHER GENES (*kat, gapA-1, dsbA, etc*.)

Previously unrecognized antigens are discovered by microarrays



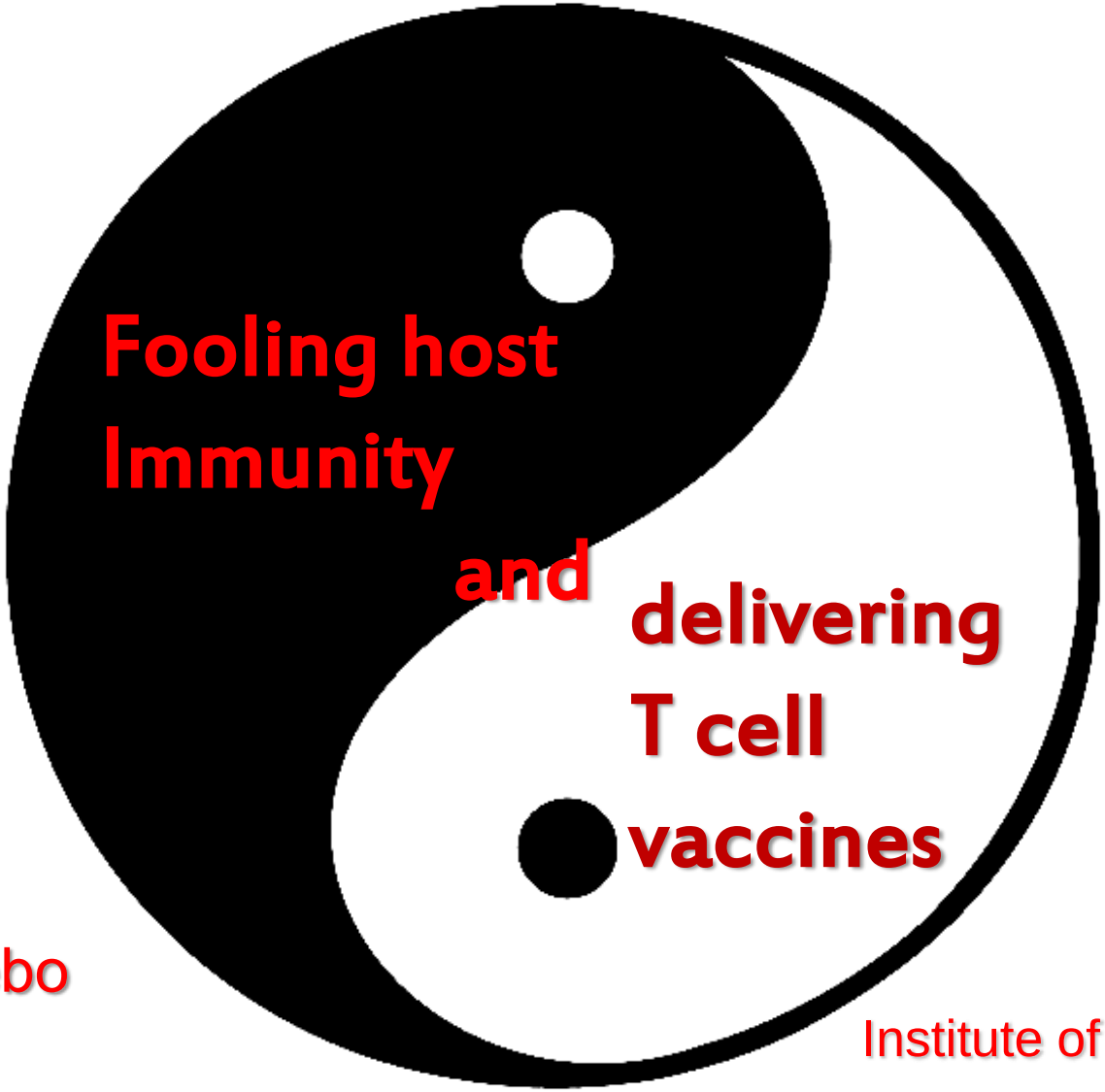
CONCLUSIONS 2

1. ADHESION INDUCES A SUBSTANTIAL MODIFICATION IN MenB MEMBRANE COMPONENTS

2. DNA MICROARRAYS COUPLED TO FACS ANALYSIS USING BACTERIAL PROTEIN ANTISERA IS AN EFFECTIVE APPROACH TO FOLLOW MEMBRANE RE-MODELING

3. AVAILABLE ALGORITHMS ARE NOT INFALLIBLE, AND PROTEIN COMPARTIMENTALIZATION HAS TO BE EXPERIMENTALLY CONFIRMED

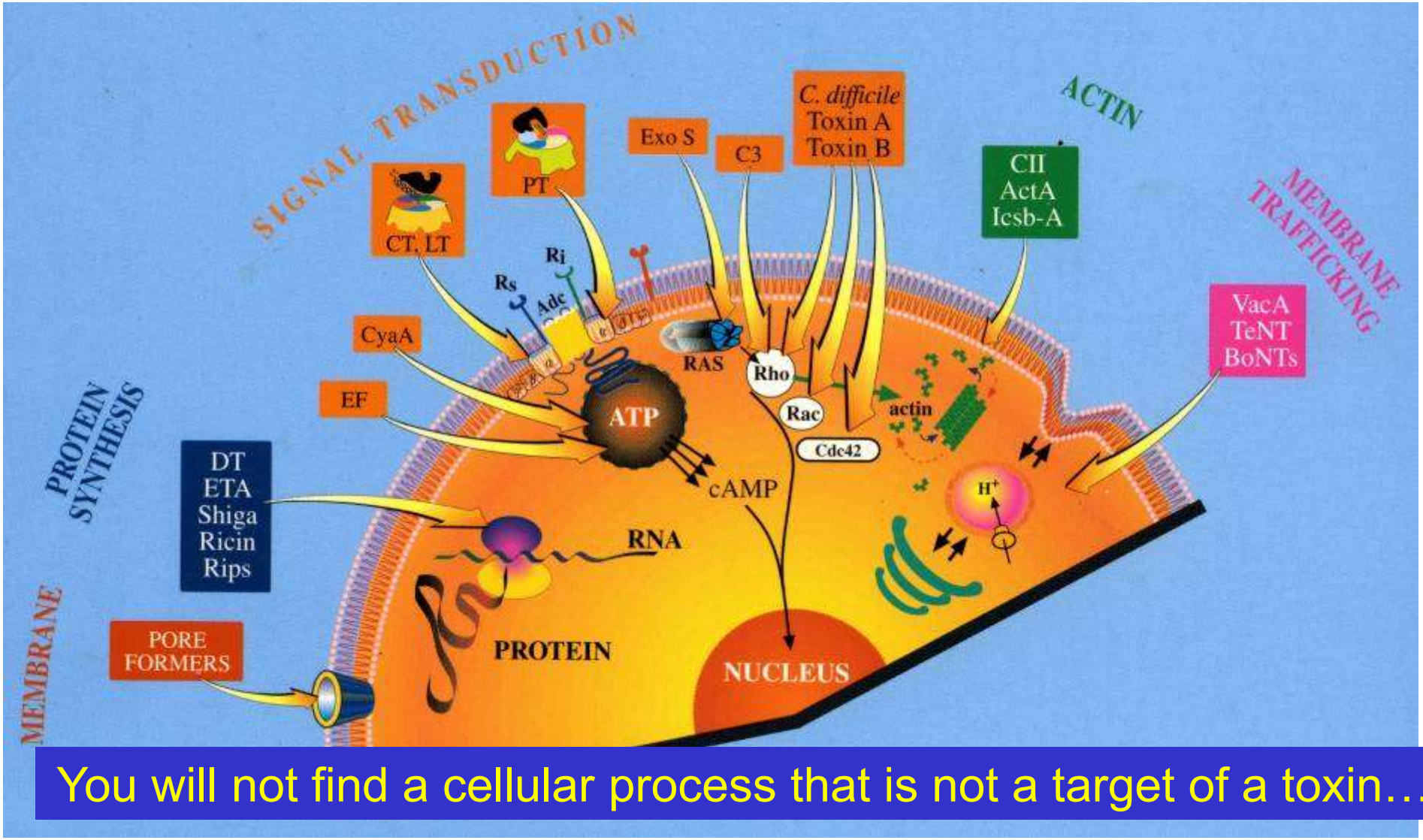
THE YIN AND YANG OF A BACTERIAL TOXIN



P. Šebo

PRAGUE
Institute of Microbiology

Bacterial protein toxins are “smart, pretty and useful”

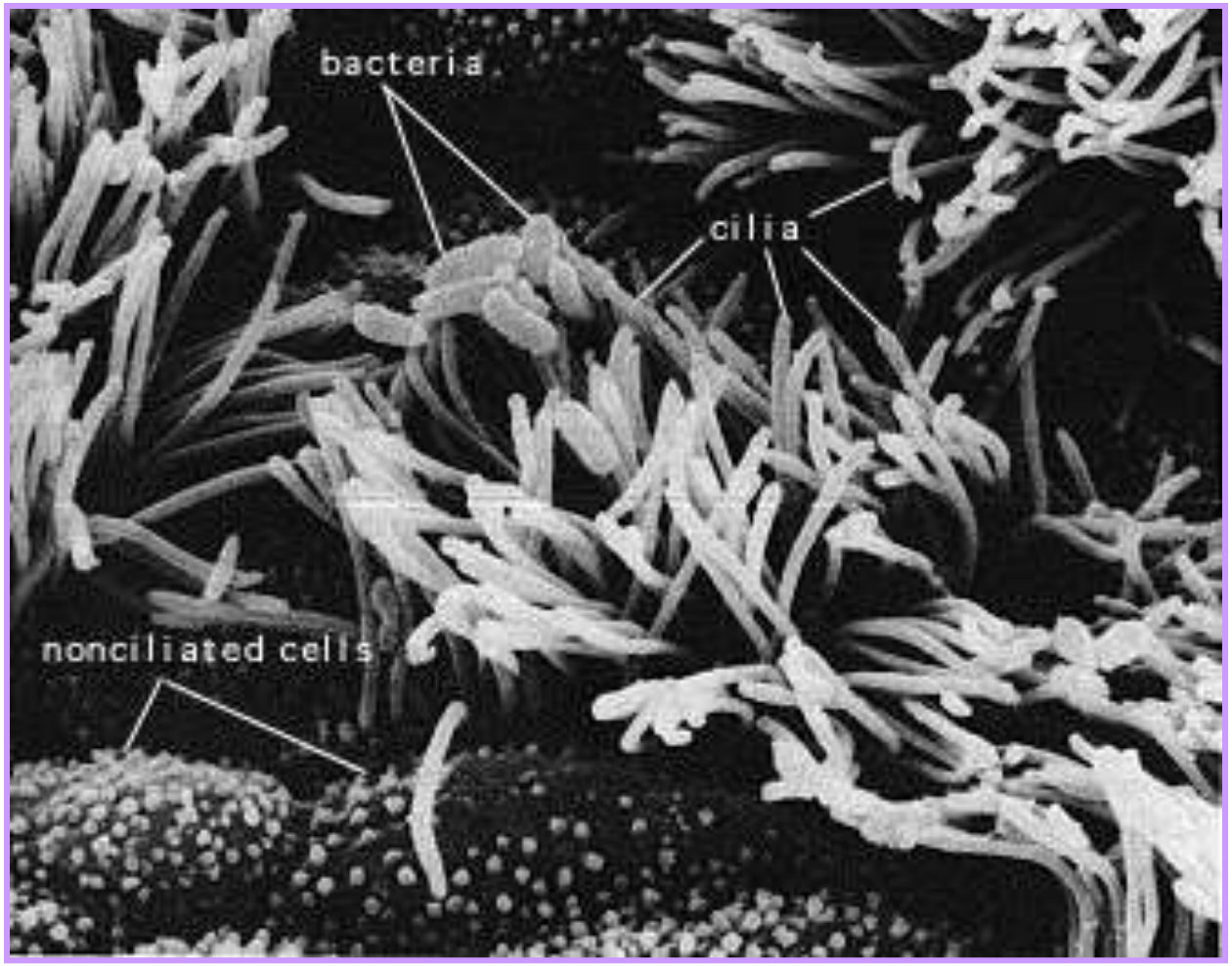


You will not find a cellular process that is not a target of a toxin…

So:

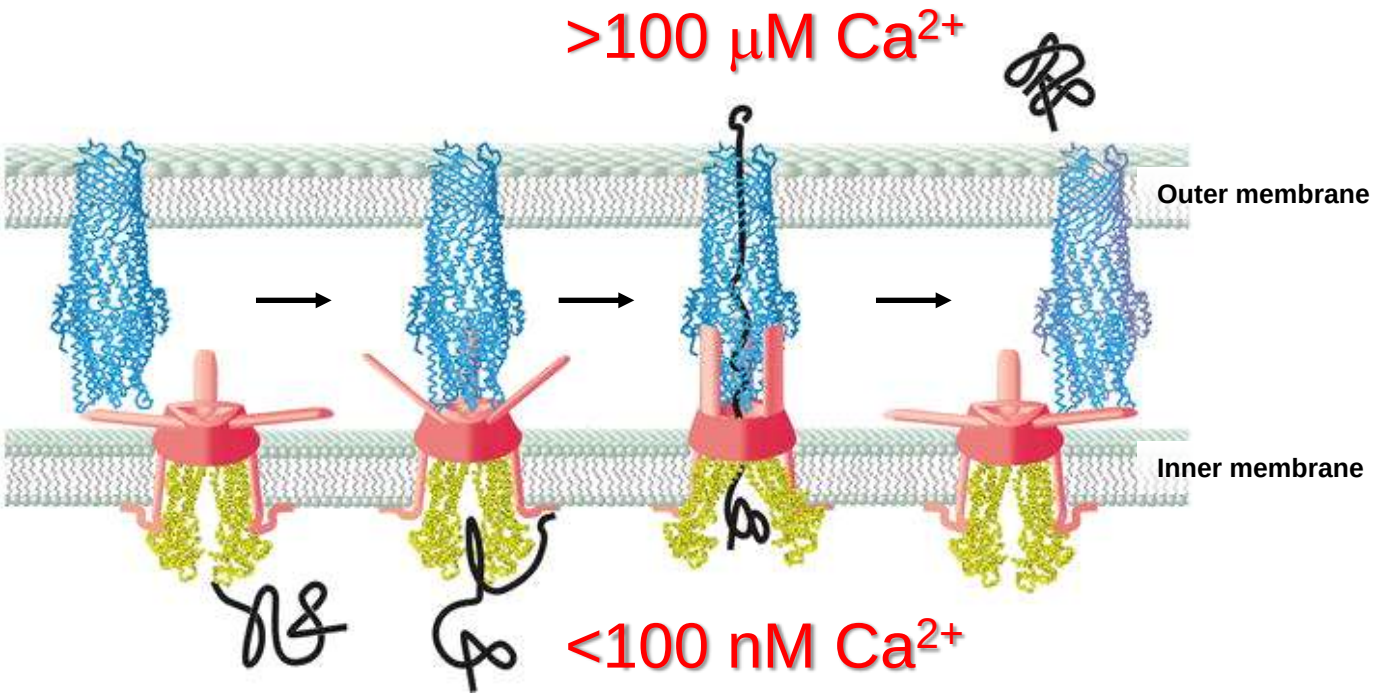
Drugs from Bugs…

Without Adenylate cyclase toxin *Bordetella pertussis* is avirulent



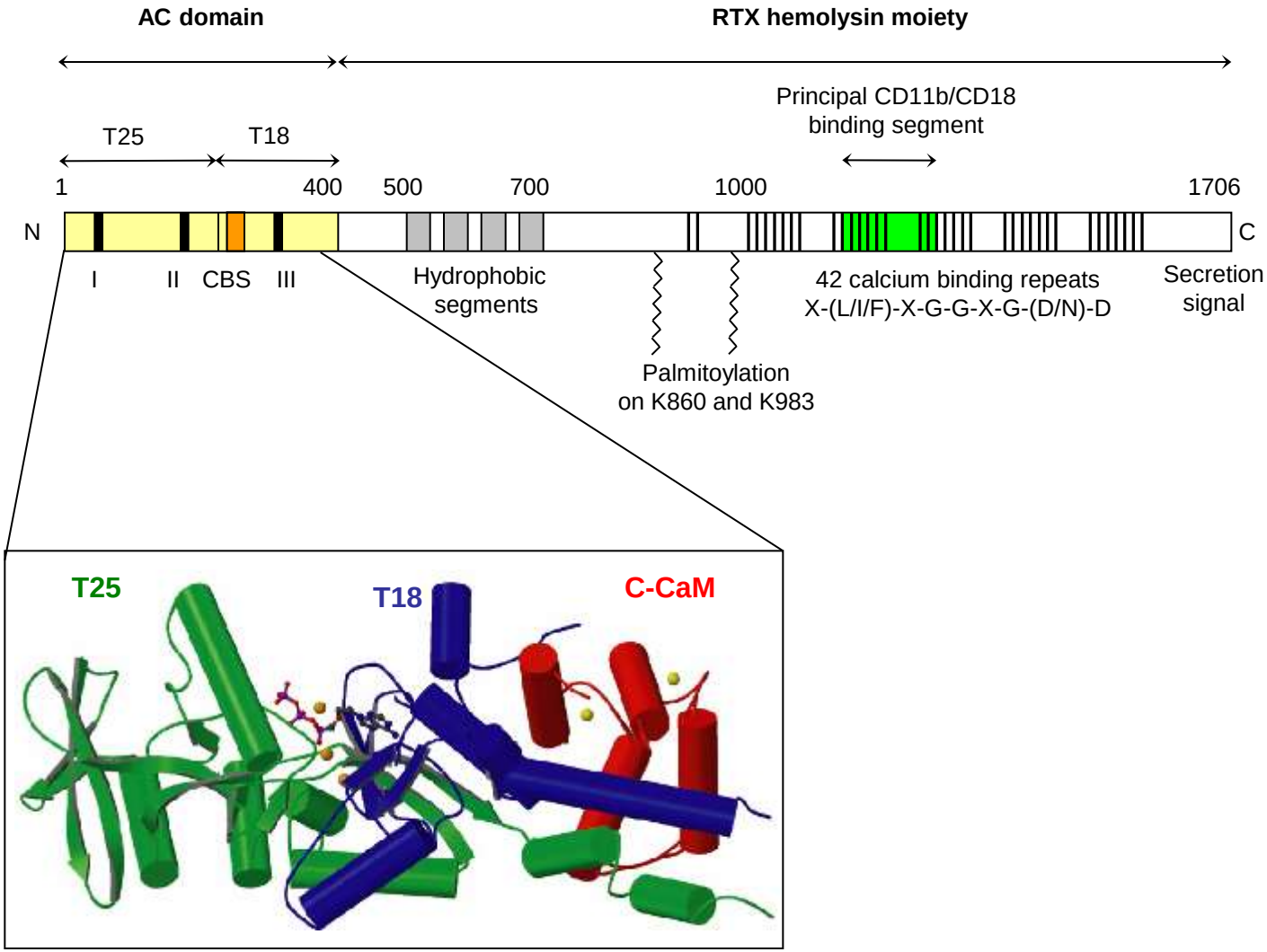
Colonisation of respiratory epithelium by *B. pertussis*
www.textbookofbacteriology.net

RTX proteins are secreted by a type I system…



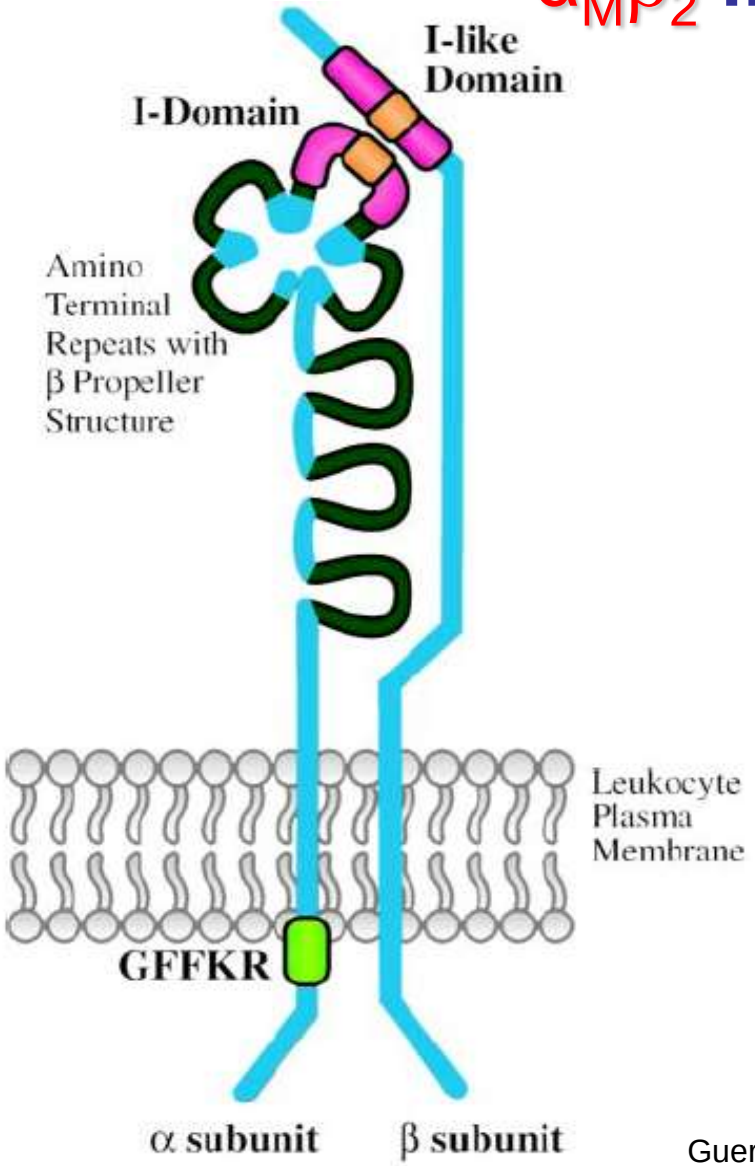
Need to unfold and refold
on the way to target…

Adenylate cyclase toxin - cytolysin



Guo Q. *et al.* (20005) EMBO J. 24, 3190–3201

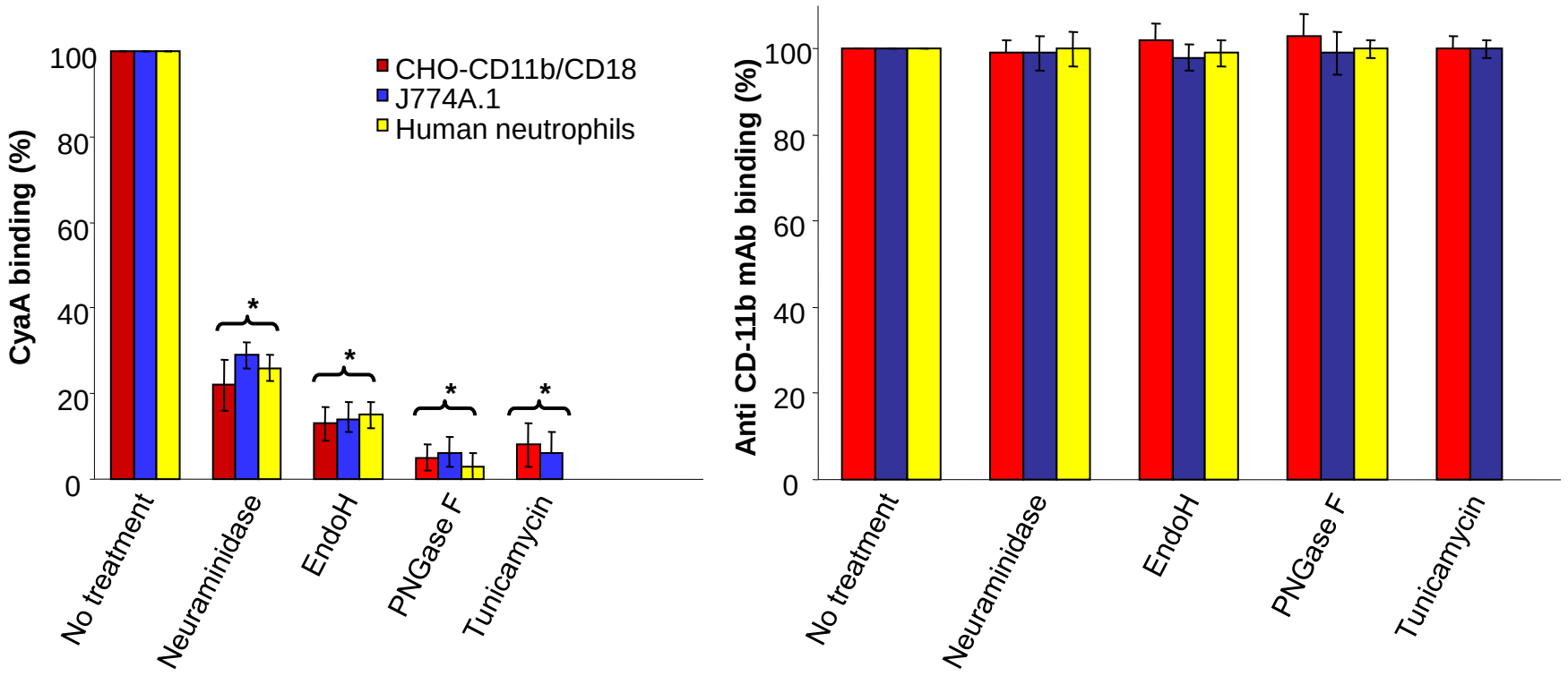
ACT targets myeloid phagocytes bearing $\alpha_M\beta_2$ integrin CD11b/CD18



- β_2 subfamily
- complement receptor 3 (CR3), Mac-1, Mo-1, $\alpha_M\beta_2$
- monocytes, granulocytes, macrophages, NK cells, neutrophils and **dendritic cells**, certain B cell subtypes

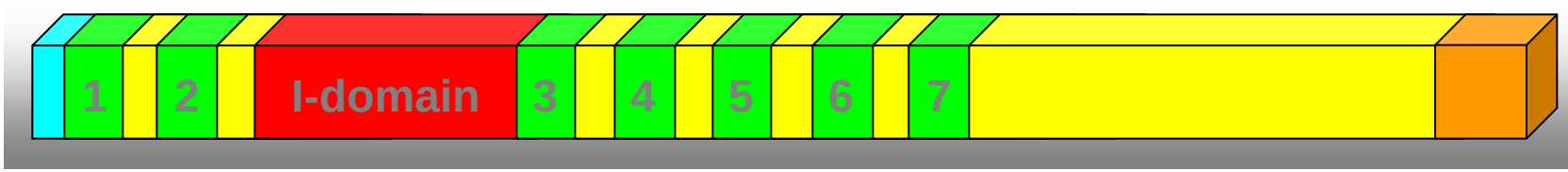
Guermonprez et al. 2001, J Exp. Med.

ACT also is a lectin…
binding of ACT to CD11b^{+} is decreased by deglycosylation



Morova et al. (2008) PNAS 105, 5355

Construction of CD11c harboring residues 342-424 and/or 614-682 replaced with homologous segments of CD11b



CD11b

X

CD11c

CD11c342-424CD11b

342 424

CD11c614-682CD11b

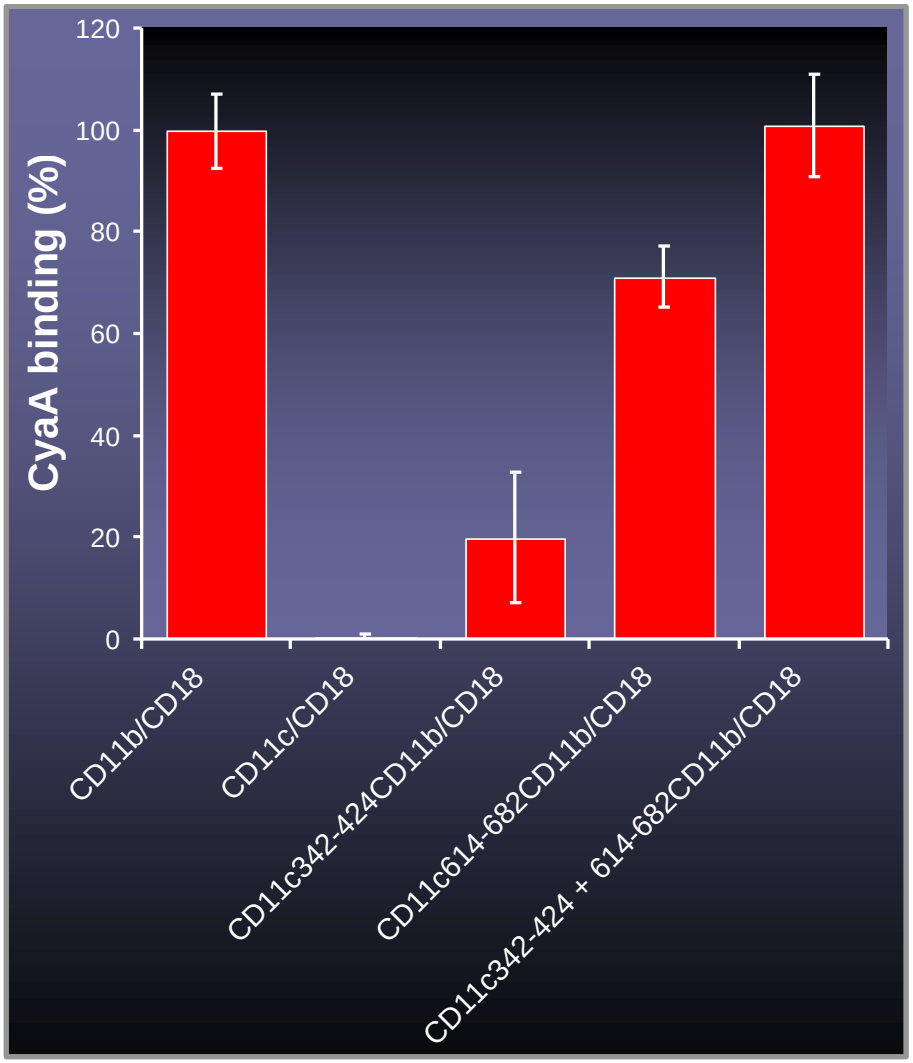
614 682

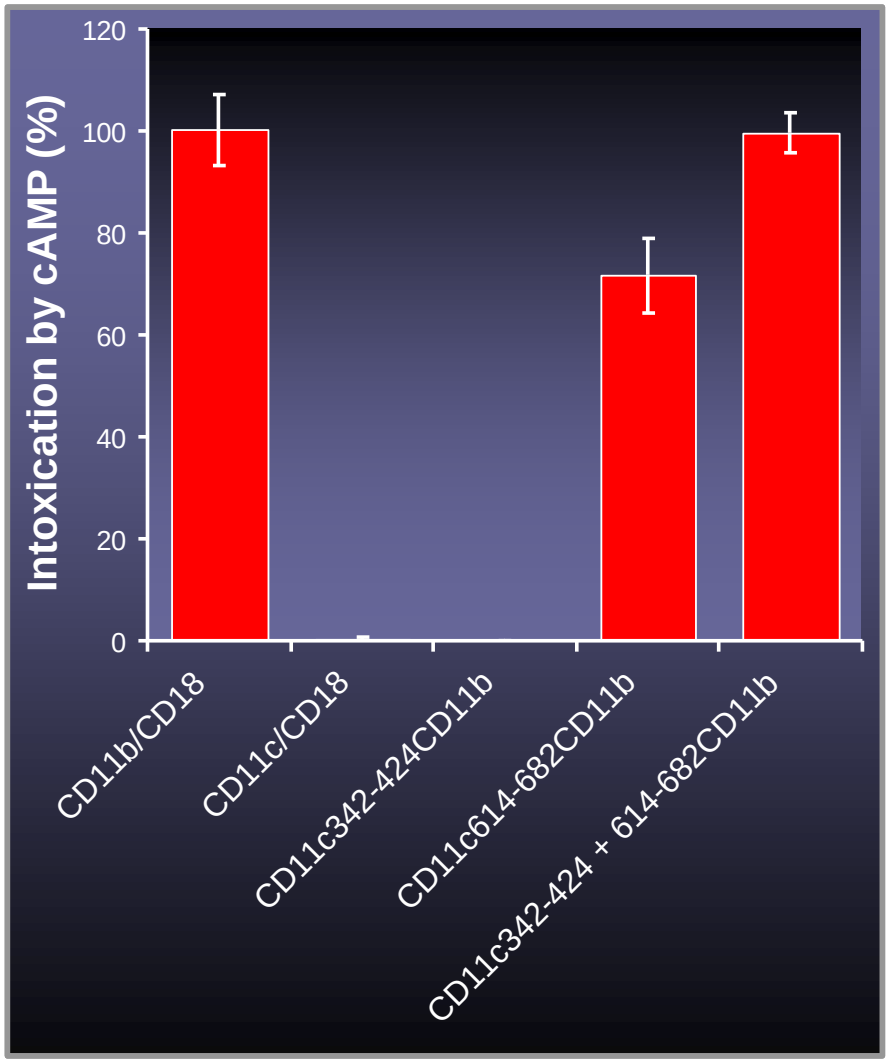
CD11c342-424+ +614-682CD11b

342 424 614 682

Radim Osička

Combination of residues 342-424 and 614-682 of CD11b is sufficient for the full binding capacity of CyaA and intoxication of target cells by cAMP

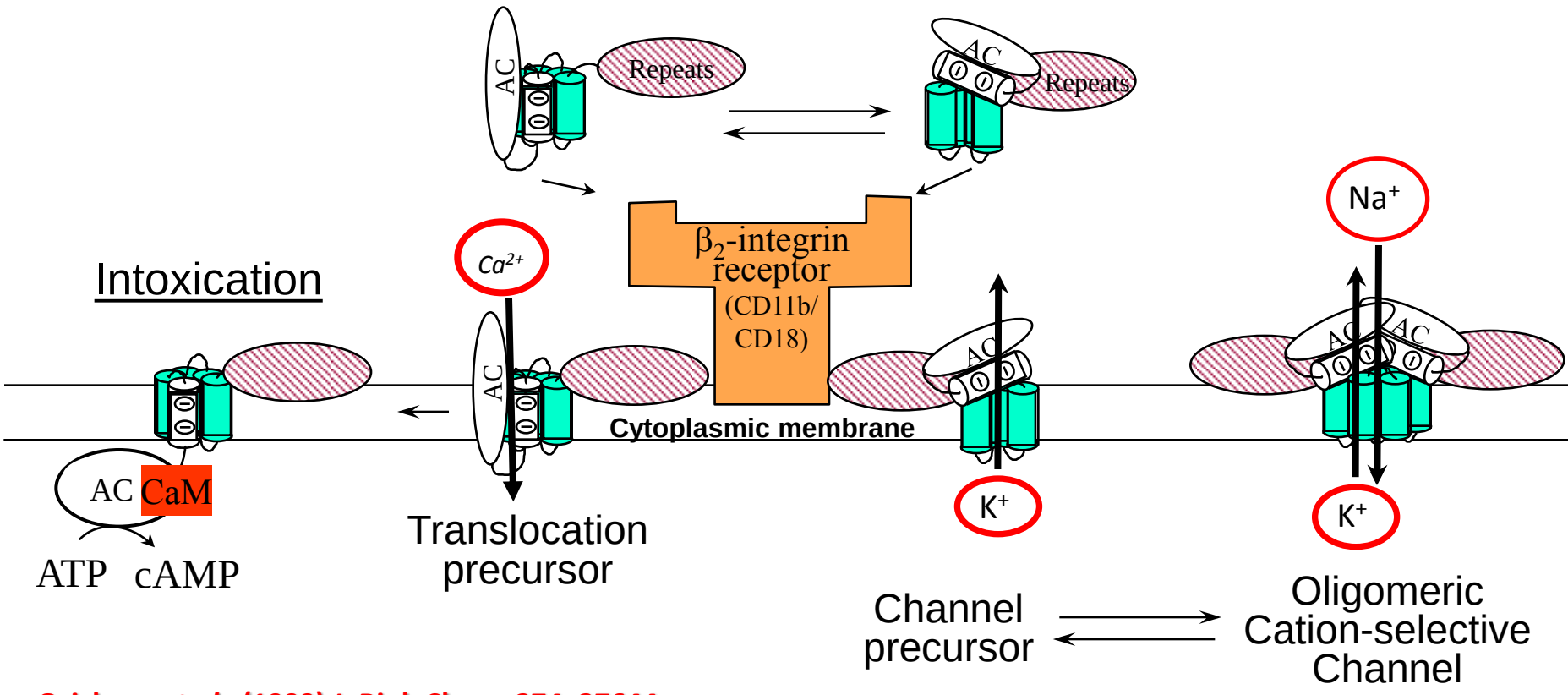




Radim Osička

Selectivity of ACT for the given β2 integrin is dictated by two CD11b-specific segments …

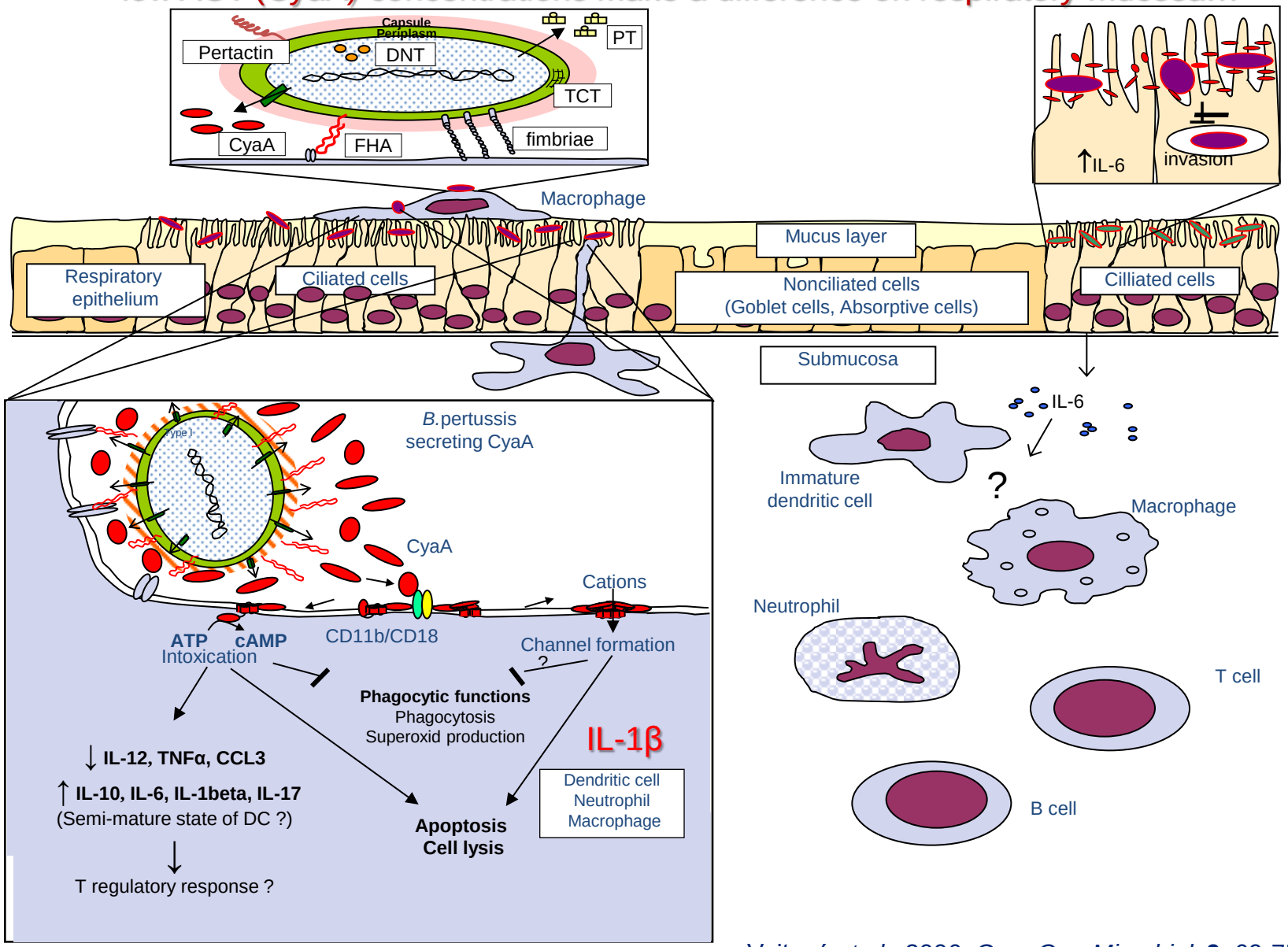
Mode of action of ACT adenylate cyclase toxin – **Cytolysin**



Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*
Basler et al., (2007) J. Biol. Chem. 282, 12419

the Yang: ACT as a SWIFT SABOTEUR

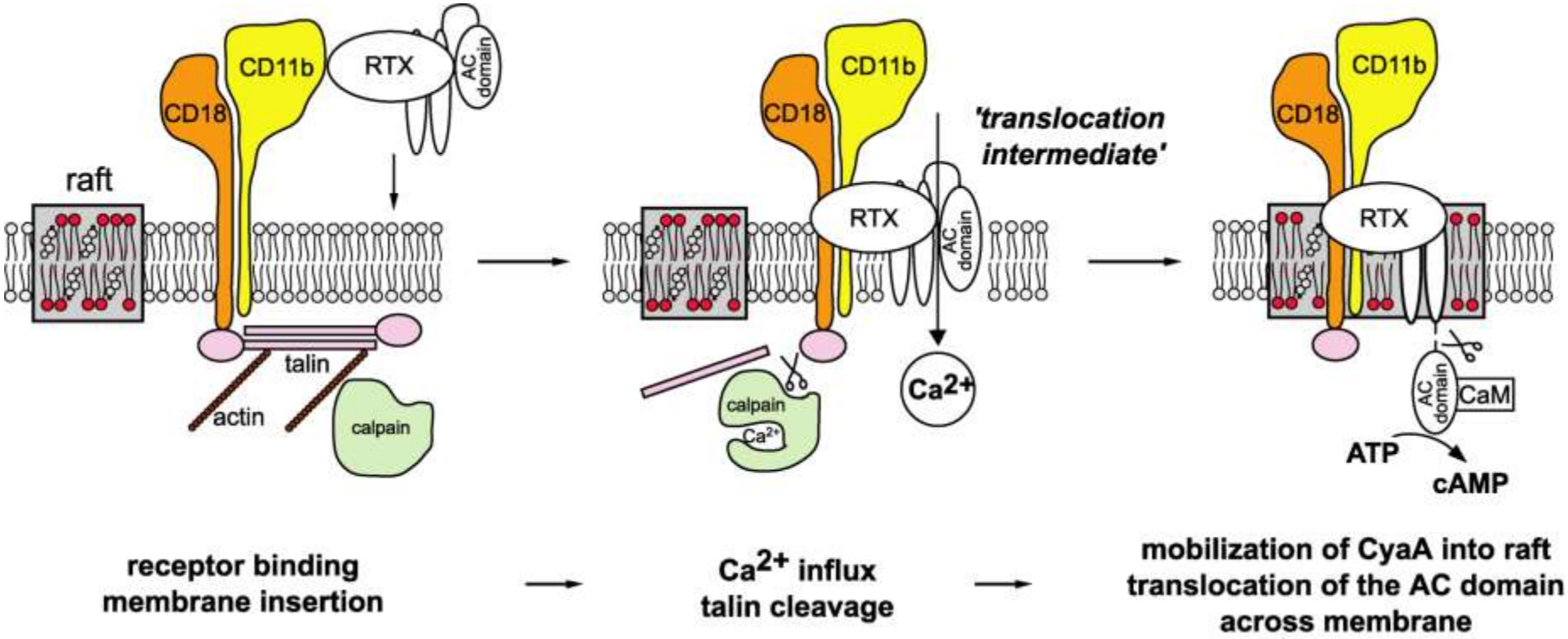
low ACT (CyaA) concentrations make a difference on respiratory mucosa…



Osičková *et al.,* (1999) J. Biol. Chem. **274**, 37644)

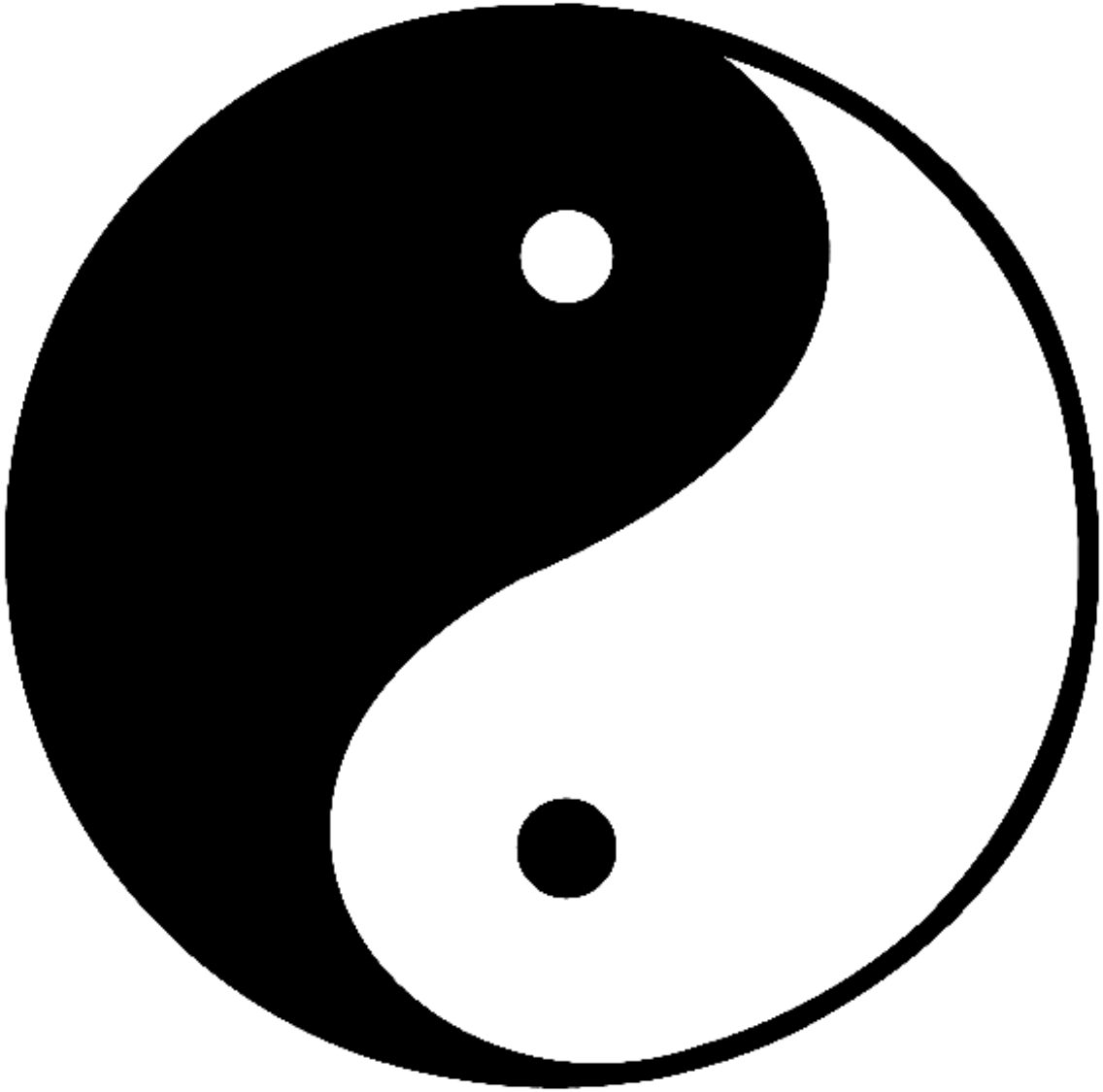
Vojtová *et al*., 2006, *Curr. Op.. Microbiol.* **9**, 69-75

Bordetella adenylate cyclase toxin hijacks its β_2 integrin receptor into lipid rafts to accomplish membrane translocation in two steps



Bumba et al. (2010). PLoS Pathog 6(5): e1000901.

THE YIN
OF A BACTERIAL TOXIN"



Make ACT to a tool of the immunologist:

- ACT targets the $\alpha_M\beta_2$ integrin CD11b/CD18

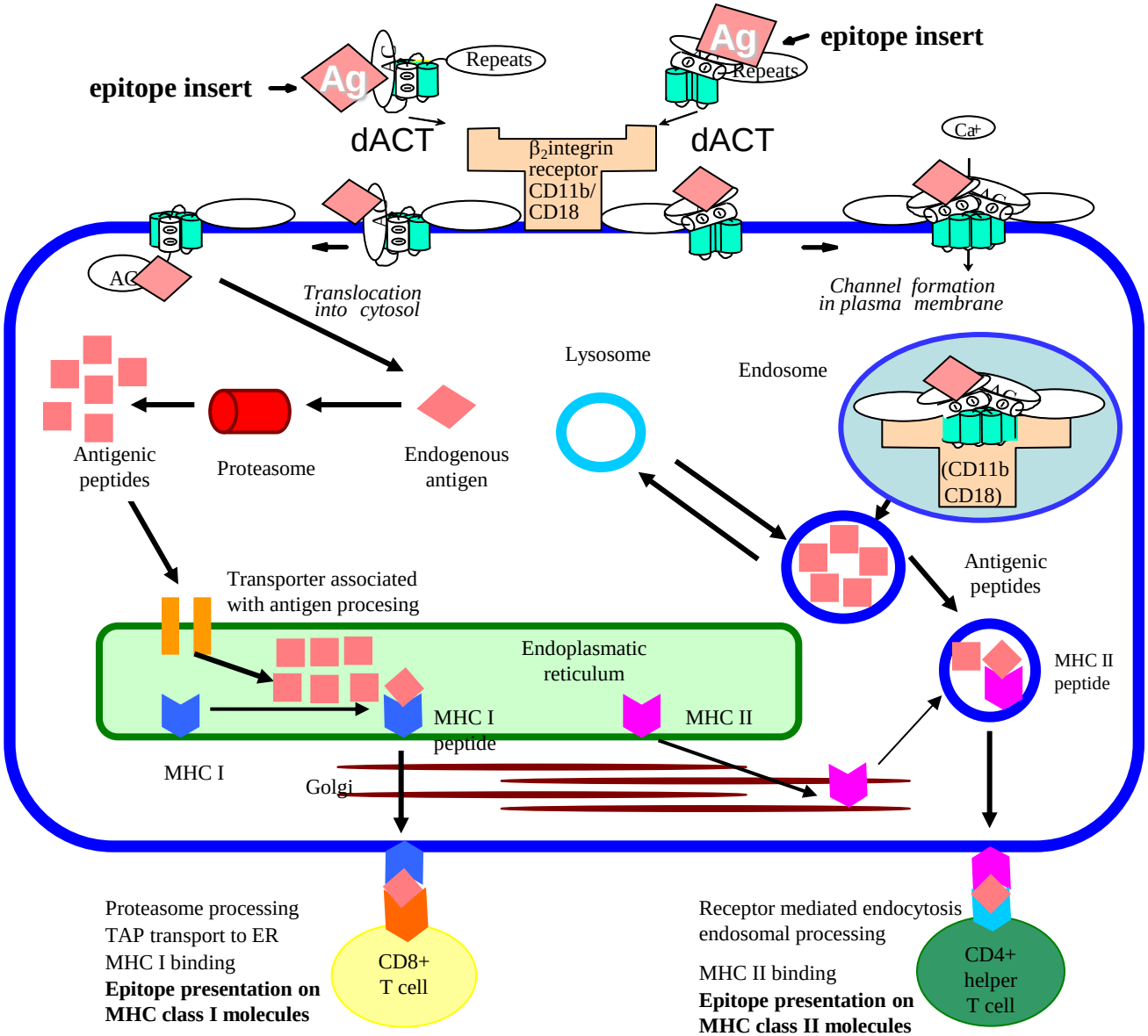
  specifically present on professional antigen presenting cells:

  - dendritic cells
  - macrophages

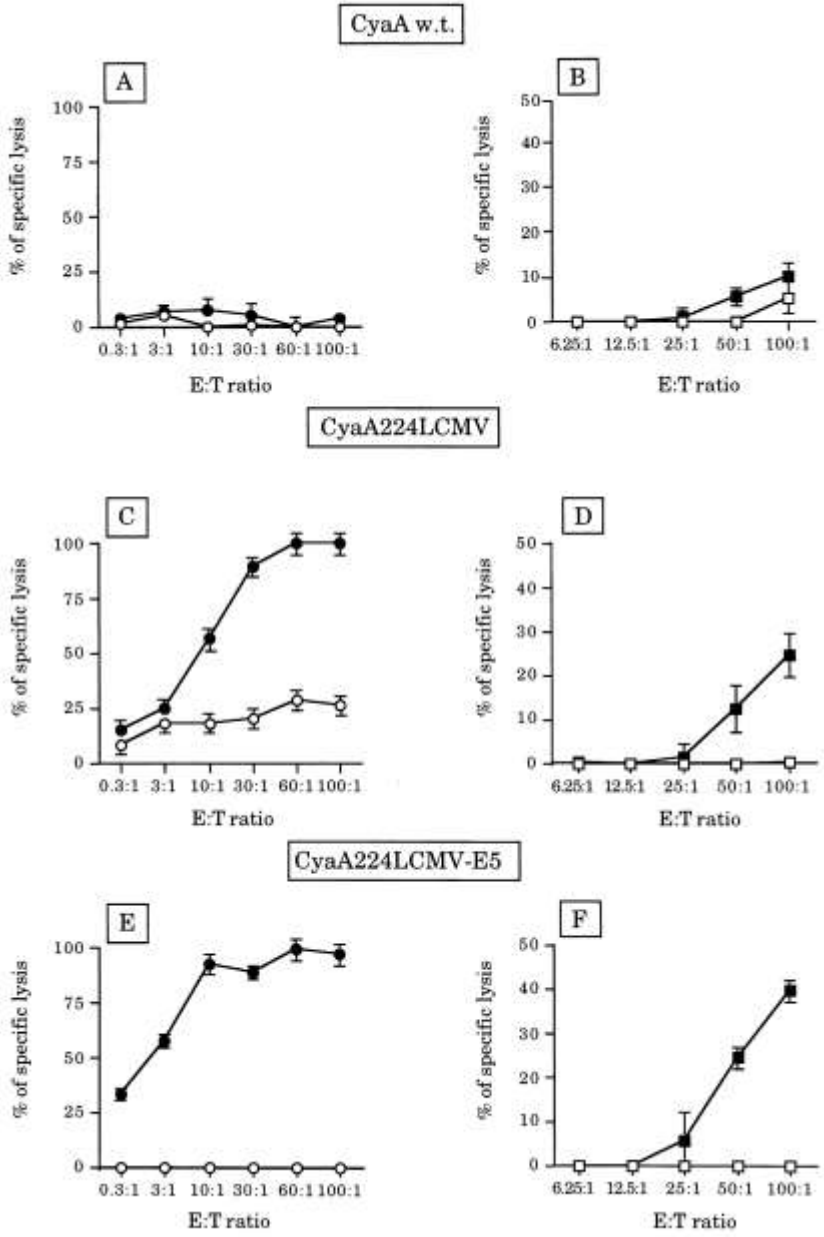
- Use ***DETOXIFIED* dACT- AC^-** to a novel tool for antigen delivery to dendritic cells:
  - –for vaccination against infections
  - –Immunotherapy of certain tumors
  - –diagnostics of infections and cancer

Exploit for antigen delivery to DCs…

dACT as a novel antigen delivery tool

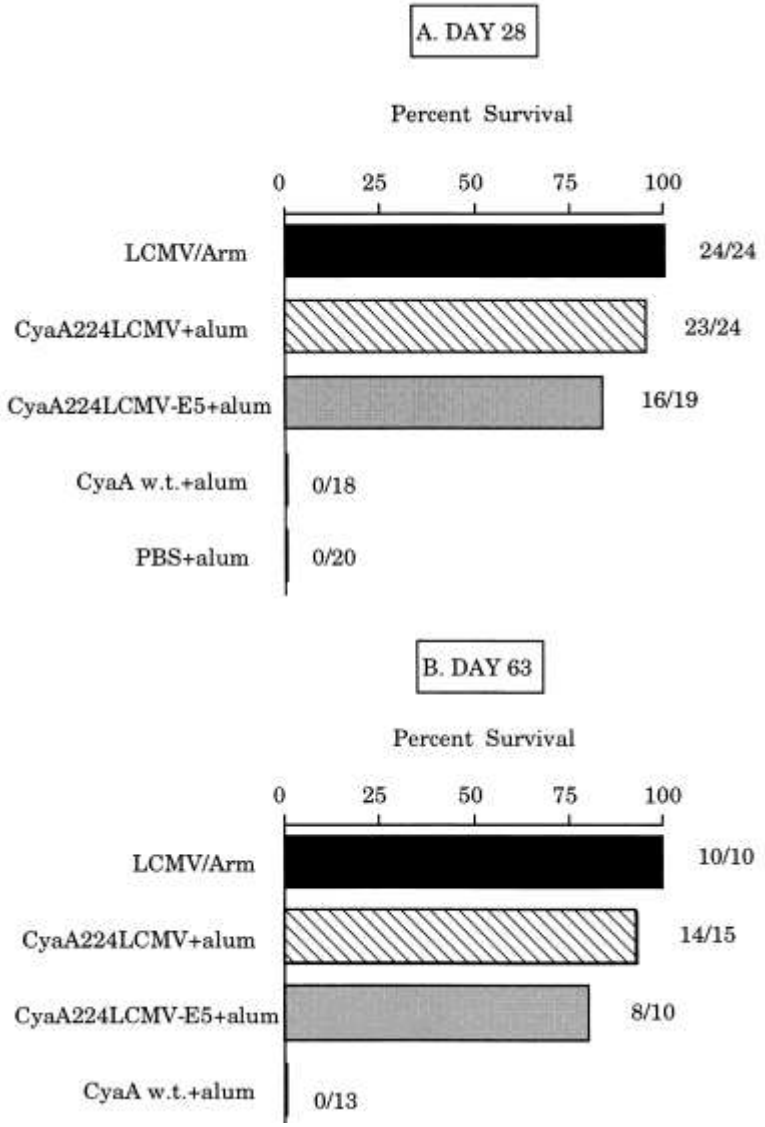


Antigen delivery by dCyaA allows induction of specific CTL reponses



Saron, M. F. et al. (1997) Proc. Natl. Acad. Sci. USA 94, 3314-3319

Immunization with CyaA-LCMV affords protection against a lethal challenge by LCMV



Saron, M. F. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 3314-3319

dCyaA constructs allow induction of

POLYVALENT

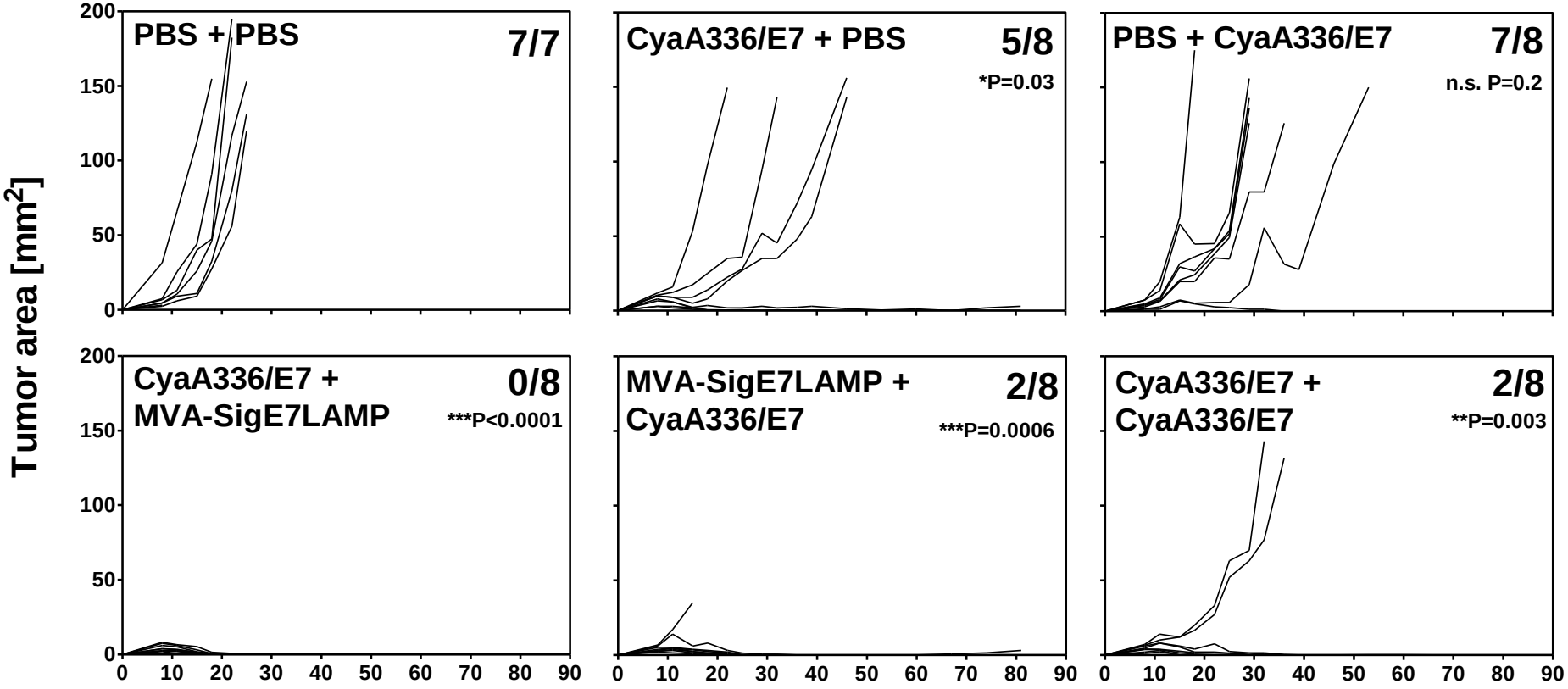
CD8^{+} CTL responses

Insertion point	CTL induction
SSLAHG^{107} VR-*V3-LCMV-OVA*-VH ^{108}HTAVDL	+++
LKEYIG^{335} VR-*V3-LCMV-OVA*-VH ^{336}QQRGEG	+++
SEATGG^{232} VR-*V3-LCMV-OVA*-VH ^{233}LDRERI	+++

Mice are protected against an LCMV challenge

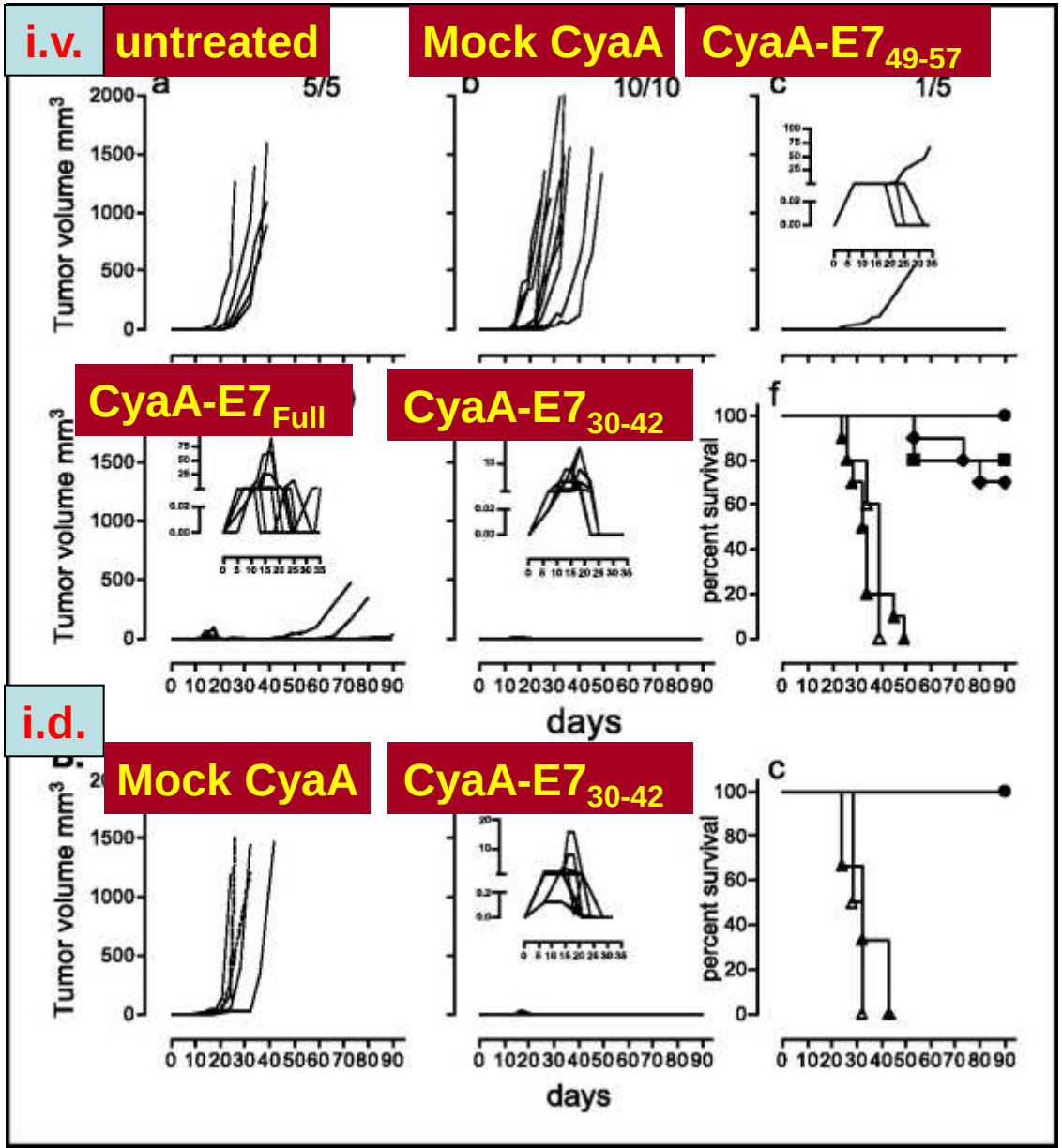
Fayolle *et al.* (2001) *J. Virol.* 75, 7330-8

Prime/Boost Immunotherapy of HPV16-induced tumors by combimations of CyaA-E7 and MVA-E7 vaccines (higher challenge dose)



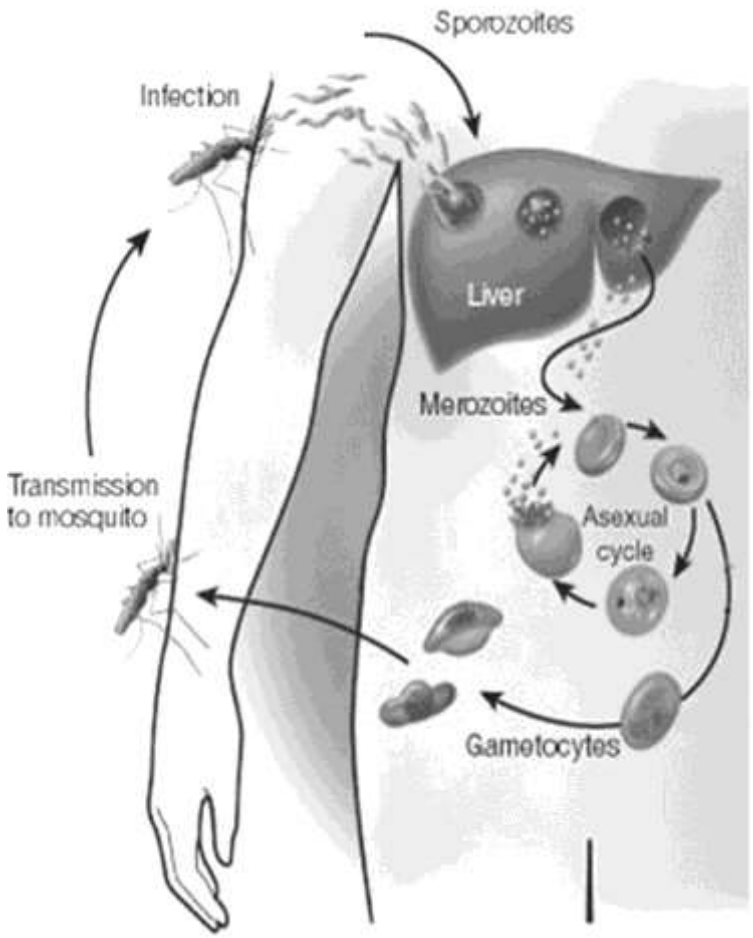
Mackova *et al.* (2006) Cancer Immun. Immunother.55, 39-46

Therapeutic vaccination with recombinant HPV16-E7 CyaAs allows eradication of established tumors and mice survival



Preville *et al.(2005)*
Cancer Res. 65, 641-649

Experimental vaccination against malaria



liver stage → **circumsporozoite protein (CSP)**

- **few days, asymptomatic replication of the parasites within hepatocytes**

→ blocking antibodies against sporozoites

→ CTL response against infected hepatocytes

blood stage

- **replication of the parasites within the erythrocytes**
- **causes pathology of the infection**

→ blocking antibodies against merozoites and gametocytes

Induction of protective immunity against mouse malaria

Mice immunized with:	infected	% protection
PBS	10/10	0%
α-CTLA-4	5/5	0%
ACT-CSP	9/9	0%
ACT-CSP + α-CTLA-4	4/10	**60%**

→ **prime/boost immunisation with ACT-CSP does not induce protective immunity**

→ **blockade of CTLA-4 during boost immunisation leads to significantly enhanced protection against P. berghei challenge**

Tartz *et al.*, 2006, *Infect. Immun.*

dACT allows induction of antigen-specific T cell responses

$CD8^+$ Antigens	$CD4^+$ Antigens	
OVA	**MalE**	Sebo *et al*., 1995, *Infect. Immun*.
LCMV	**MAGE**	Fayolle *et al*., 1996, *J. Immunol*.
Apa, Cfp	**ESAT-6, CFP10**	Saron *et al*., 1997, *Proc. Natl. Acad. Sci. U.S.A.*
gp120	**TB-10.4**	Osicka *et al*., 2000, *Infect. Immun*.
E7	**Ag85A**	Fayolle *et al*., 2001, *J. Virol*.
CSP		Loucka *et al*., 2002, *Infect. Immun*.
Melanoma tyrosinase		Schlecht *et al.,* 2004, *J. Immunol.*
ESAT-6, CFP10		Mackova *et al*., 2006, *Cancer Immunol. Immunother.*
TB-10.4		Tartz *et al*., 2006, *Infect. Immun*.,
		Wilkinson *et al.* 2005 *Infect Immun.*
		Anderson *et al.* 2006 *Am. J. Crit. Care Resp. Med.*
		Majlessi *et al*., 2006, *Infect. Immun*.,
		Hervas-Stubs *et al.,* 2006, *Infect Immun*

HIV, LCMV *in vitro*
HIV
LCMV *in vivo*
permisive sites
polyvalent CTL response
$CD4^+$ T cell response
Mechanisms
Tumor immunotherapy
protection against malaria
Improvement of LTBI detection
Improvement of LTBI detection
IFNγ and immunity against MTB
IFNγ and immunity against MTB

GENTICEL has selected the prevention of cervical cancer as the medical target to demonstrate the safety and efficacy of its unique therapeutic vaccine platform, the Adenylate Cyclase (CyaA)

The strategic goal of the company is to ensure that a vaccine solution can be offered to all women in order to prevent cervical cancers.

Preventive HPV vaccines are indicated for individuals, mainly teenage girls and young women, who have not yet been exposed to oncogenic Human Papillomavirus (HPV). However, these prophylactics are not effective once one is already infected (Hildesheim et al., 2007; Hung et al., 2008) and because at any given time, approximately 13% of sexually active women bear HPV (De Sanjosé et. al., 2007), Genticel is developing products that are part of a new class of vaccines, 'therapeutic vaccines', which remain active after infection and therefore complement the current preventive vaccines.

For more information go to our web page "THE HUMAN PAPILLOMAVIRUS (HPV)"

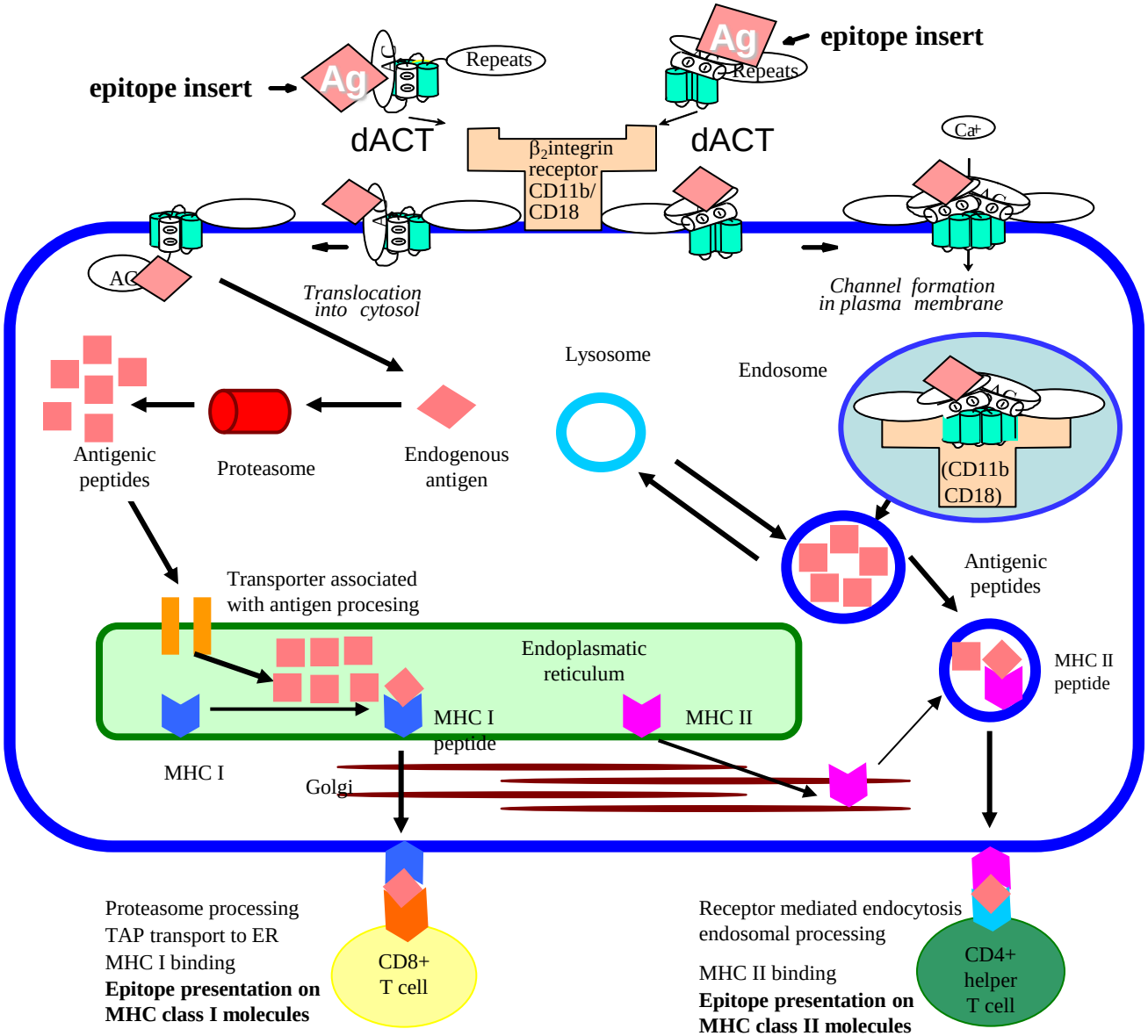
News

- **Approval for clinical trial of ProCervix**
  July, 23 2010
  Genticel's therapeutic vaccine, ProCervix, aimed at preventing cervical cancer in patients already infected by human papillomavirus (HPV), receives clearance to start a Phase I clinical trial.
  Press release

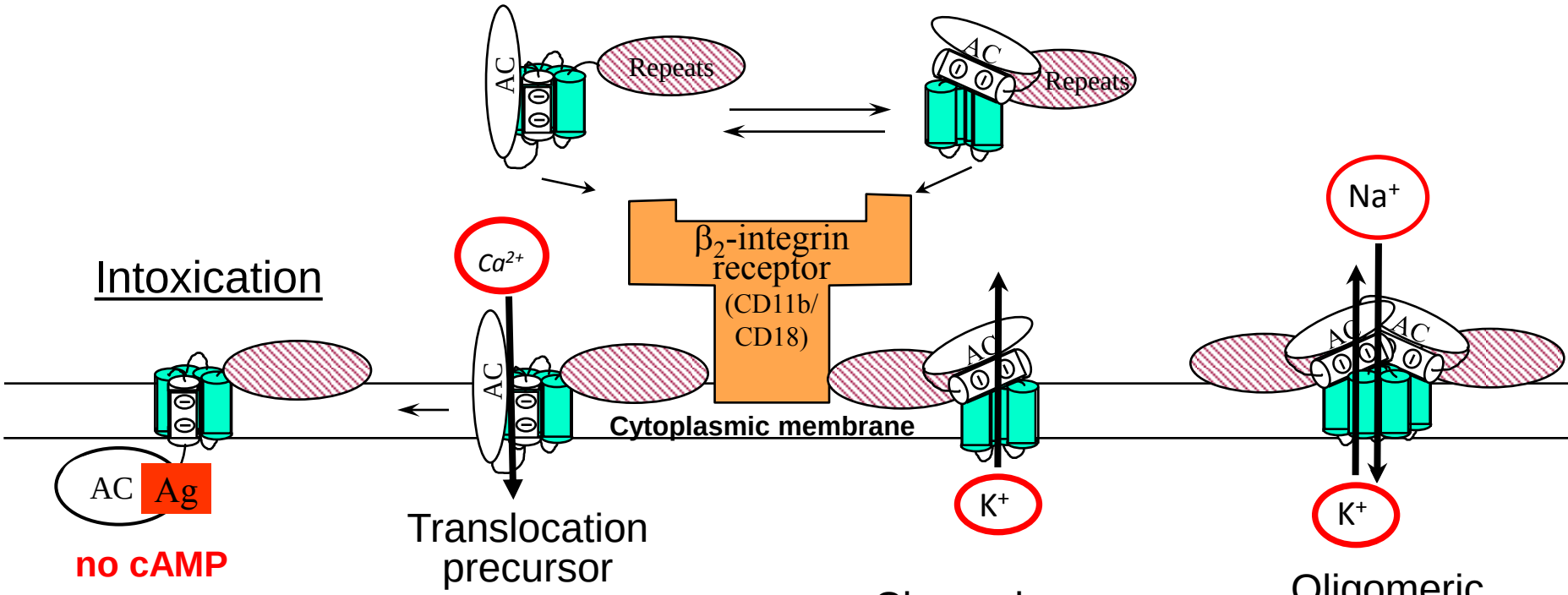
- **Le vaccin ProCervix autorisé en essai clinique**
  July, 23 2010
  Genticel annonce que ProCervix, son vaccin thérapeutique destiné à prévenir le cancer du col de l'utérus chez les patientes infectées par le virus du papillome humain (HPV), a reçu l'autorisation d'entrer en essai clinique de Phase I.
  Communiqué de presse

- **Genticel secures EUR 13.1M (USD 17.7M) in capital funding**
  March, 09 2010
  Press Release

dACT as a novel antigen delivery tool



Even the AC^{-} toxoid can exhibit immunomodultory activity through calcium signaling, cell permabilization and inflammasome activation and other?...

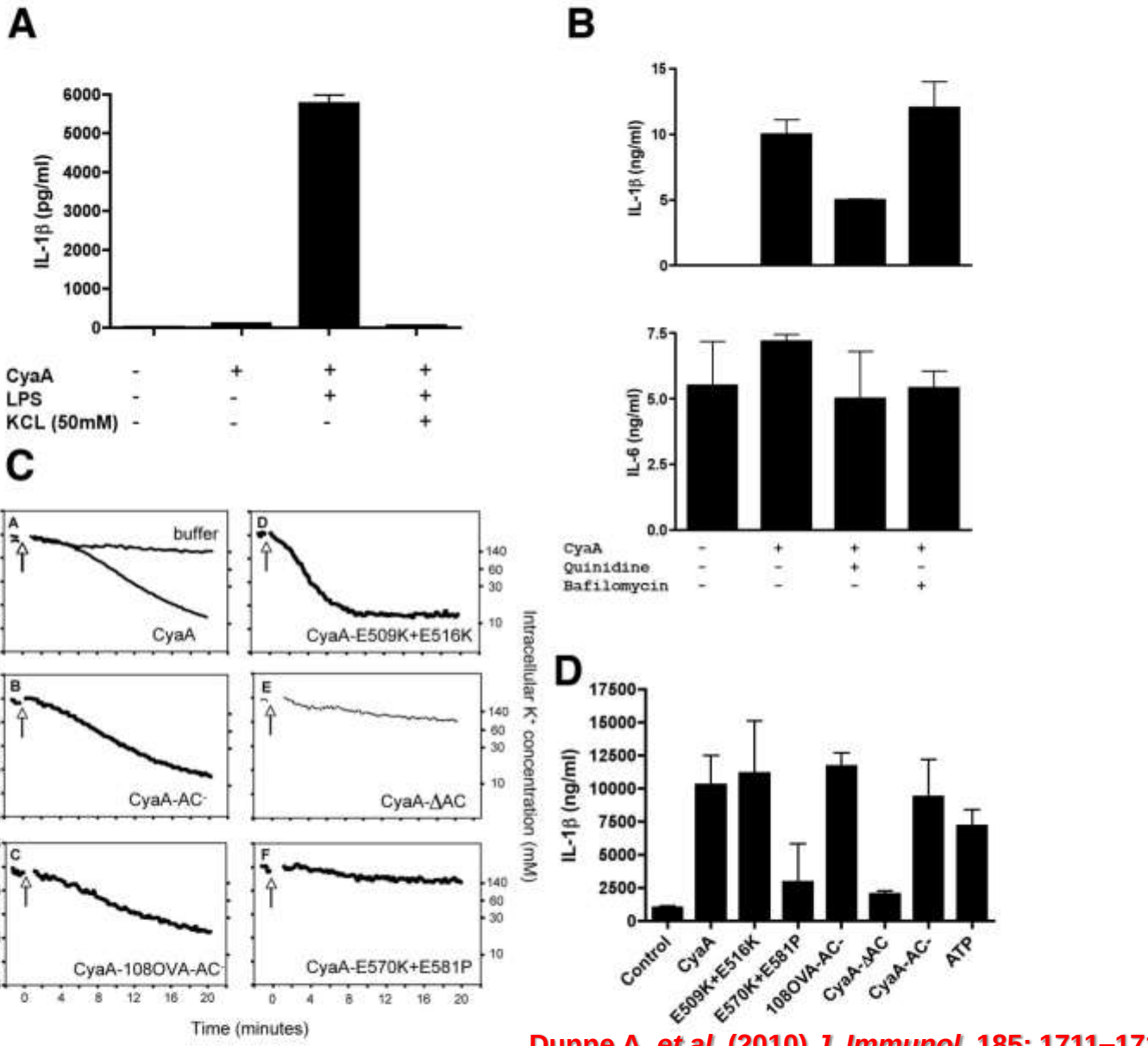


Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Osickova *et al.* (2010) *Mol. Microbiol. 75:15450-1562*

Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*

Dunne *et al.* (2010) *J. Immunol. 2010,* 185: : 1711–1719

Inflammasome activation by ACT is dependent on pore formation and potassium efflux, not cAMP



Dunne A. *et al.* (2010) *J. Immunol.* 185: 1711–1719

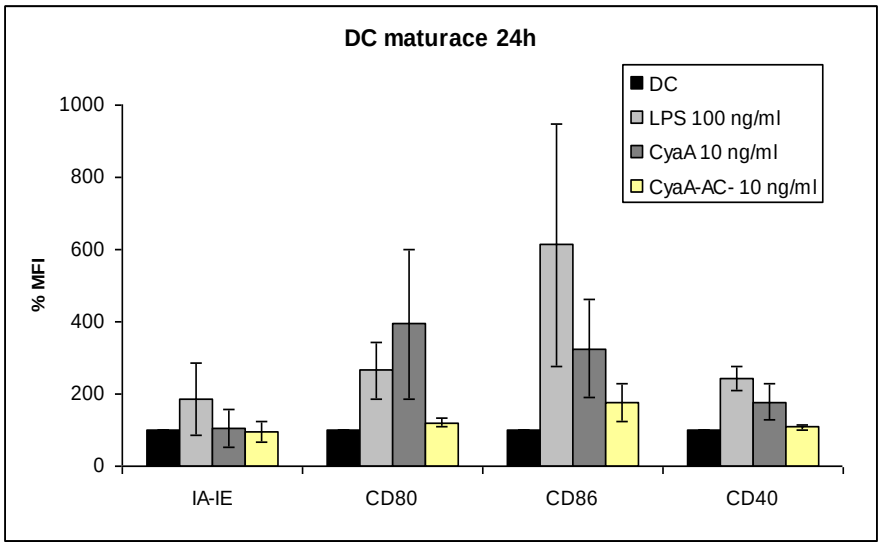
Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β–dependent adaptive immunity against tumors

The therapeutic efficacy of anticancer chemotherapies may depend on dendritic cells (DCs), which present antigens from dying cancer cells to prime tumor-specific interferon-γ (IFN-γ)–producing T lymphocytes. …dying tumor cells release ATP…triggers the NOD-like receptor family, pyrin domain containing-3 protein (NLRP3)-dependent caspase-1 activation complex (‘inflammasome’), allowing for the secretion of interleukin-1β (IL-1β).
The priming of IFN-γ–producing CD8+ T cells by dying tumor cells fails in the absence of a functional IL-1 receptor 1 and in Nlpr3-deficient (Nlrp3–/–) or caspase-1–deficient (Casp-1–/–) mice …. the NLRP3 inflammasome links the innate and adaptive immune responses against dying tumor cells.

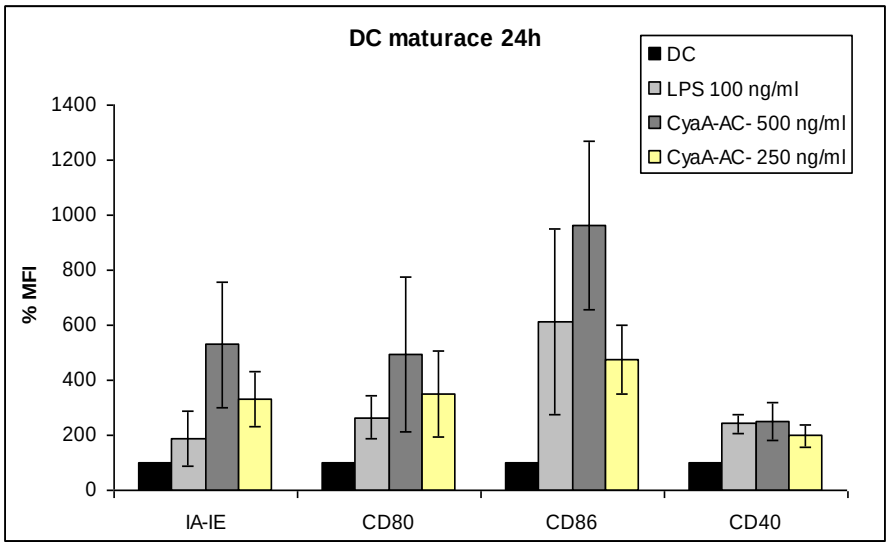
Ghiringhelli and ….. Laurence Zitvogel (2009) *Nature Medicine* 15, 1170-1179

CyaA *versus* CyaA-AC$^-$ induced maturation of BMDC: costimulatory molecules

CyaA 10 ng/ml



CyaA-AC- 250 ng/ml



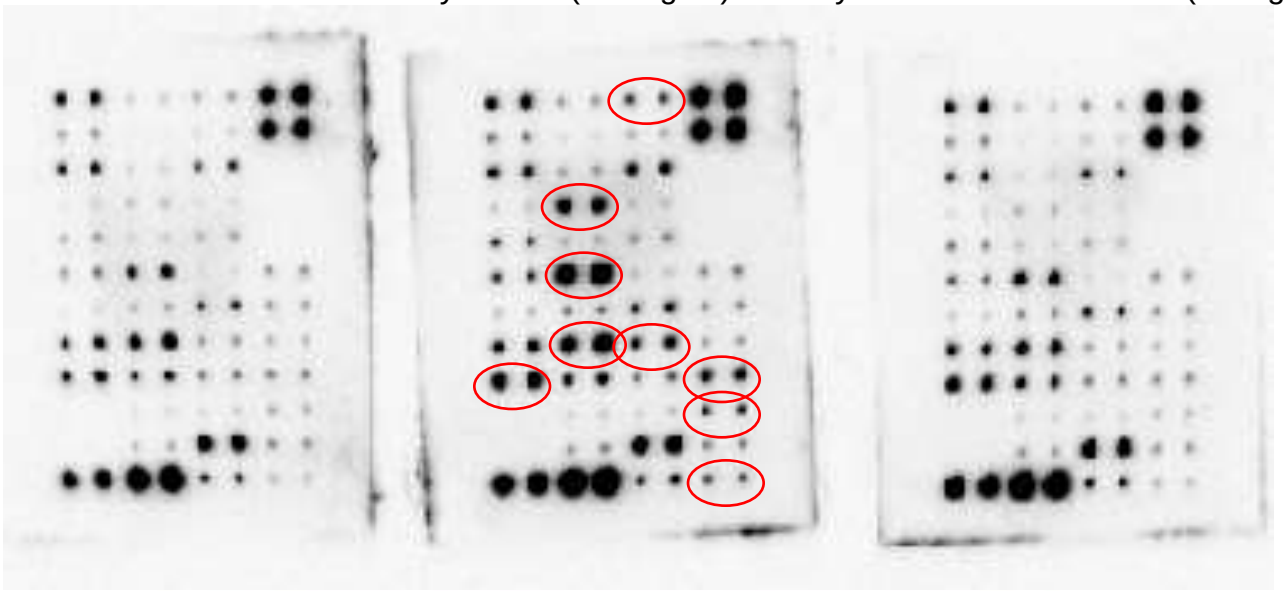
- cAMP effect
- phenotypic maturation of DC
- but proinflammatory cytokines missing! (IL-12, TNF-α)
- induction of tolerogenic T cells ?

- Ca^{2+} influx ⟶ signalling in DC ?
- K^+ efflux ⟶ activation of inflammasome ?
- toxoids for immunization
- importance of adjuvant effect

Cytokine / chemokine profile

stimulation ↑ **Mouse inflammation antibody array** ↓ inhibition

DC | CyaA-AC- (300 ng/ml) | CyaA-E570K+E581P-AC- (300ng/ml)



IL-6 proinflammatory cytokines
KC (IL-8)
LIX
MCP-1 chemokines
Eotaxin-2
GM-CSF differentiation factors
GCSF
sTNF R II
Fas Ligand

RayBio® Mouse Inflammation Antibody Array 1 (40)

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	Blank	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
2	POS	POS	NEG	NEG	Blank	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
3	GM-CSF	IFNγ	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
4	GM-CSF	IFNγ	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
5	IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	MCP-1	MCSF	MIG	MIP-1α	MIP-1γ
6	IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	MCP-1	MCSF	MIG	MIP-1α	MIP-1γ
7	RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNFα	sTNF RI	sTNF R II	Blank	Blank	POS
8	RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNFα	sTNF RI	sTNF R II	Blank	Blank	POS

n=1

Current status of dACT-antigen delivery technology

1997 - Protective immunity against a virus (LCMV)

1999 - Immunotherapy of transplanted tumors in mice

2004 - Enhanced detection of latent tuberculosis

2005 - Protective immunity against *Plasmodium* (mouse malaria model)

2005 - immunotherapy of experimental tumors (such as HPV16 – induced)

(*US Patent* No. 5,503,829, No. 5,679,784, No. 5,935,580, *EU Patent application* No. 03291486.3, *US Prov.* 03495, 6094 (2003))

It flies or it dies???

Tell you next time ….

- Phase I clinical trial for HPV16/18-induced cervical carcinoma started July 7, 2010 by Genticel S.A. Toulouse France
- Phase I/II clinical trial in melanoma patients starts soon (EU 6. FP consortium THERAVAC)


Peter


Ivo


Irena


Marek
Jirka
Radim


Marcela


Jana


Jana


Zuzana


Jana


Lada


Adriana


Radek


Lenka


Ondra


Jana H


Katka


Ilona


Hanka


Soňa


Peter
the big boss...


Radim


Jirka


Marek


Radek


Katka


Irena L.


JanaV


Jiřina


JanaK


OndraČ


Kája


MartinaK


JanaH


JanaM


PavelMi


PavelMa


Tomáš


Jawid


MartinaS


Eva


Zuzana


HanaJ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