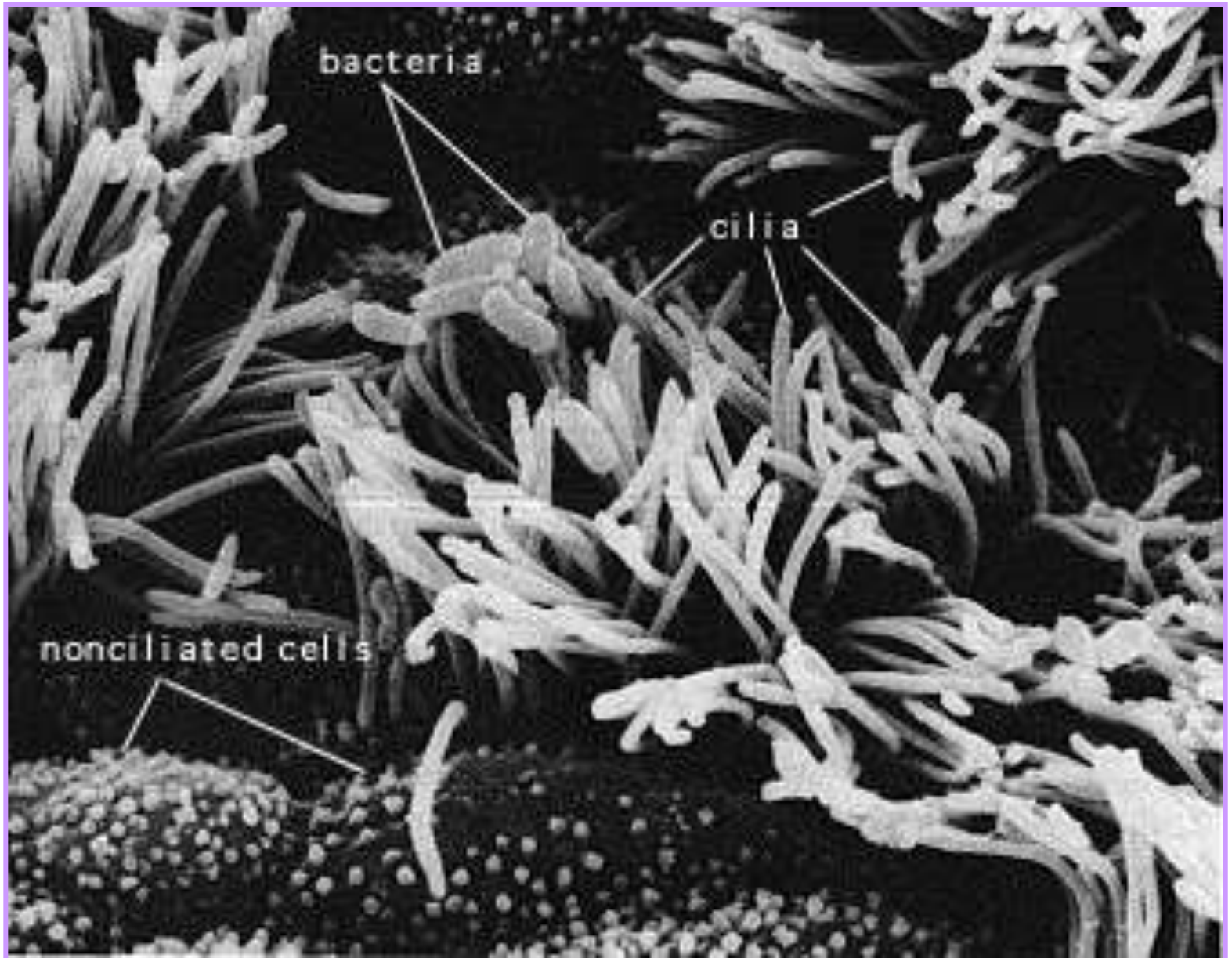
Bordetella pertussis

The agent of whooping cough (pertussis)



Whooping cough used to be a major cause of infant morbidity and death in the pre-vaccine era in CR

(slide dr. Fabianova NIPH)

Died of pertussis according to age groups in 1949-1957

Procházka J., Kryl R. Praktický lékař, 6/1959

Věk v měsících	1949	1950	1951	1952	1953	1954	1955	1956	Celkem 1949-1956	Celkem %	1957	1957 %
- 1	673	280	463	234	276	157	89	87	2259	**76,0**	114	66,0
1-2	126	49	113	45	56	46	15	25	475	**16,0**	**96%!!!**	
2-3	30	11	16	12	13	21	11	9	123	**4,1**		
3-4	15	3	8	1	6	13	4	1	51	**1,7**		
4-5	3	0	6	2	4	5	1	1	22	**0,7**		
5-9	6	4	7	0	7	7	0	1	32	**1,1**		
10-14	2	1	0	0	0	1	0	1	5	**0,2**	59	34,0
15-19	0	0	0	0	0	0	0	0	0	**0,0**		
20-24	1	0	0	0	0	0	0	0	1	**0,0**		
25-29	0	0	0	1	0	0	0	0	1			
55-59	0	0	1	0	0	0	0	0	1	**0,2**		
Nezn. věk	0	0	2	0	0	0	0	0	2			
Celkem	856	348	616	295	362	250	120	125	2972	**100,0**	173	100,0

Vaccination

- pertussis used to be a leading cause of infant death in the pre-vaccine era

DTP (1942)

- D – diphteria, T – tetanus, P – pertussis (suspension of killed bacteria)
- side effects (pain, convulsions, irreversible brain damage)

DTaP (acellular pertussis) introduced in US in 1999

- PT toxoid and an adhesins FHA and PRN + Fim2/3 eventually
- protection from symptoms, but not from colonization
- circulation of strains with less or no production of antigens present in DTaP

40 millions infected people every year, around 300 000 deaths

Bordetella pertussis

One of the few TRUE human pathogens

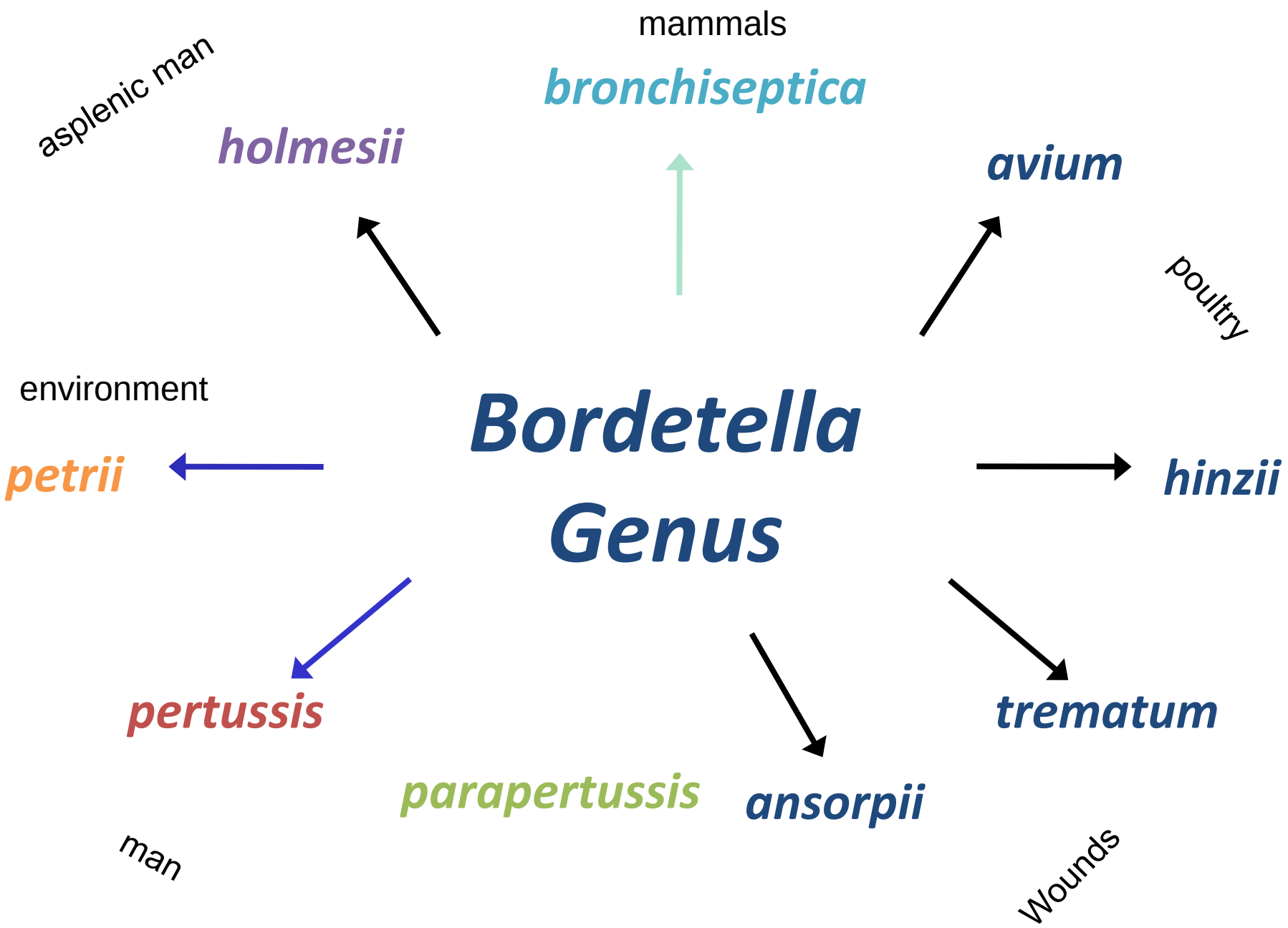
No fever!!!
Pertussis can kill nonvaccinated infants before being diagnosed

Least controlled vaccine-preventable infectious disease…

30–50 million pertussis cases/year

200 – 300 thousands deaths/year worldwide

- **Recent circulating *B. pertussis* strains adapt to acellular vaccine pressure – resurgence in US and EU**


mammals
bronchiseptica
asplenic man
holmesii
avium
poultry
environment
Bordetella
Genus
petrii
hinzii
pertussis
trematum
parapertussis
ansorpii
man
Wounds

Bordetella Genus

B. petrii, ***B. bronchiseptica*** **and *B. holmesii* are species able to infect humans, <u>mostly immuno-suppressed,</u> and**

- **persist inside the host**
- **cause bacteremia**

Is the inability of *B. parapertussis* or *B. pertussis* to persist inside their host due harnessing of the immune systém by toxins?

Bordetella Genus

Bordetella petrii
(5500 kb)

Bordetella bronchiseptica
(5338 kb)

Bordetella holmesii
(3732 kb)

Bordetella parapertussis
(4773 kb)

Bordetella pertussis
(4086 kb)

Institut Pasteur

Bordetella Genus

Bordetella petrii

(FHA like, TCT ?, LPS?)

Bordetella bronchiseptica

(FHA, PRN, Fim±, TCT, AC-Hly, BteA, no PT, Fla, LPS±)

Bordetella holmesii

(FHA like?, TCT?, LPS?)

Bordetella parapertussis

(FHA, PRN, TCT, AC-Hly, LPS, no PT)

Bordetella pertussis

(FHA, Fim, PRN, TCT, PT, AC-Hly, BteA± but no TSSIII)

Institut Pasteur

Relationships between *B. bronchiseptica*, *B. pertussis* and *B. parapertussis*$_{hu}$

Animal origin

Human origin

Complex IV *B. bronchiseptica* 5 338 kb

5.57 – 1.11

3.5 – 0.7 Complex II *B. pertussis* 4 086 kb

Complex I *B. bronchiseptica* 5 338 kb

1.4 – 0.27

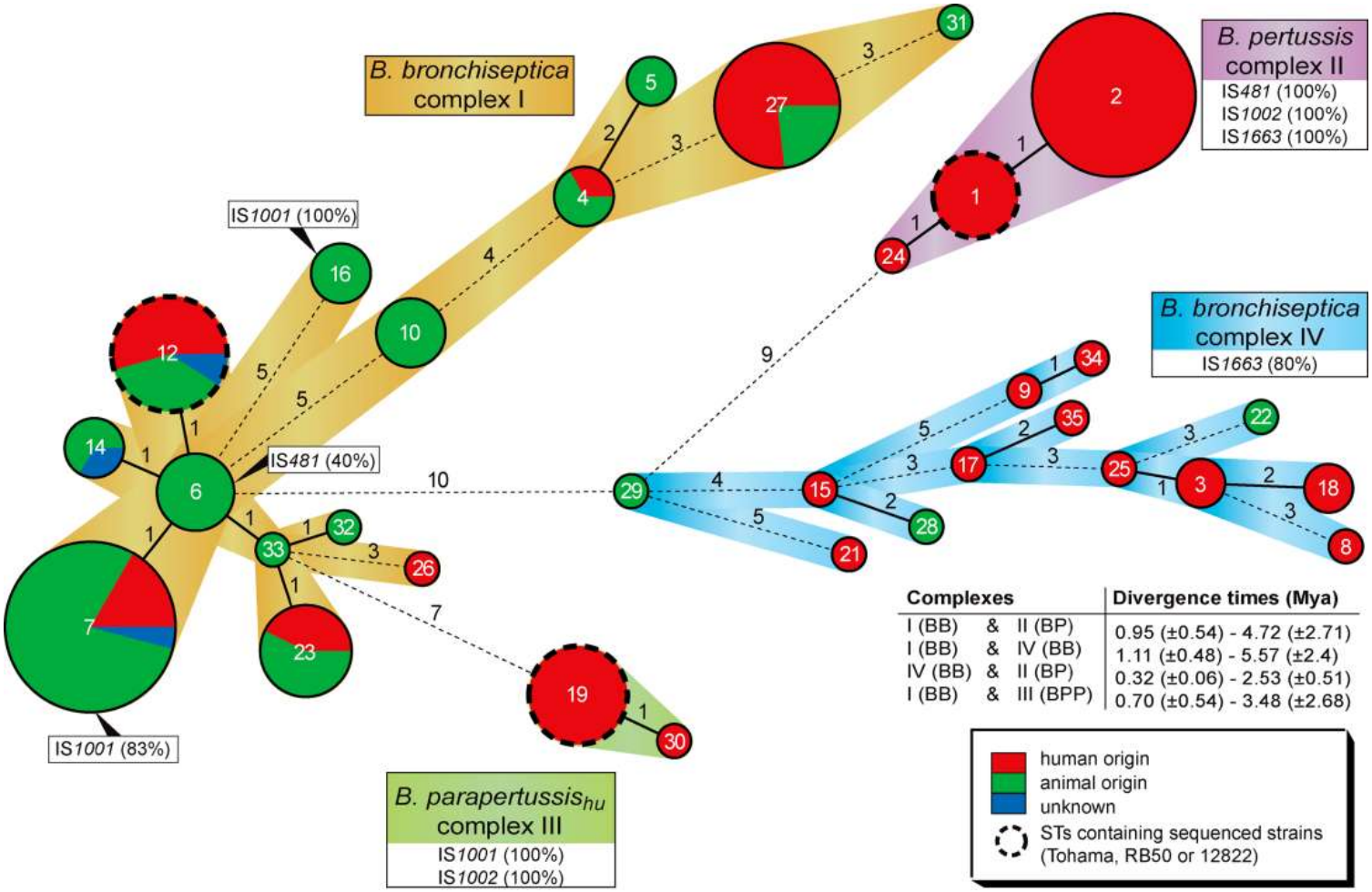
Complex III *B. parapertussis* 4 77 7 kb

5 4 3 2 1 0

Time in millions years

J. Parkhill et al. 2003 et D. A. Diavatoploulos et al. 2005

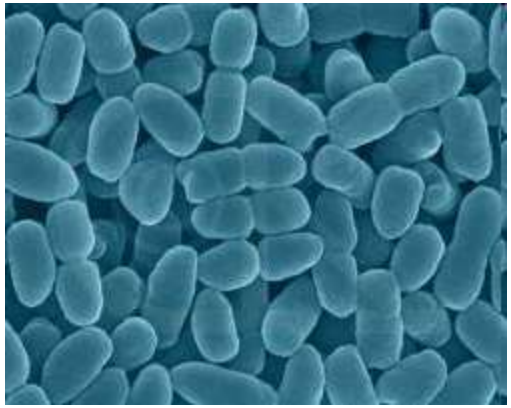
Minimum spanning tree of *B. bronchiseptica*, *B. pertussis* and *B. parapertussis*.



The tree was based on the sequence of seven housekeeping genes. Individual genes were split into five subloci, and a categorical clustering was performed. In the minimum spanning tree, sequence types sharing the highest number of single locus variants were connected first.
Each circle represents a sequence type (ST) the size of which is related to the number of isolates within that particular ST.
Colors within circles indicate host distribution. The numbers between connected STs represents the number of different subloci between those STs. The clonal complexes (I, II, III and IV) are indicated by colored strips between connected STs.
The distribution of insertion elements IS*481*, IS*1001*, IS*1002* and IS*1663* is shown in boxes;
number between parentheses indicate the percentage of strains that contained the IS as determined by PCR amplification.
The divergence times between *B. bronchiseptica* complexes I and IV and *B. pertussis* complex II are marked(Diavatopoulos *et al.*, 2005).

Bordetella pertussis

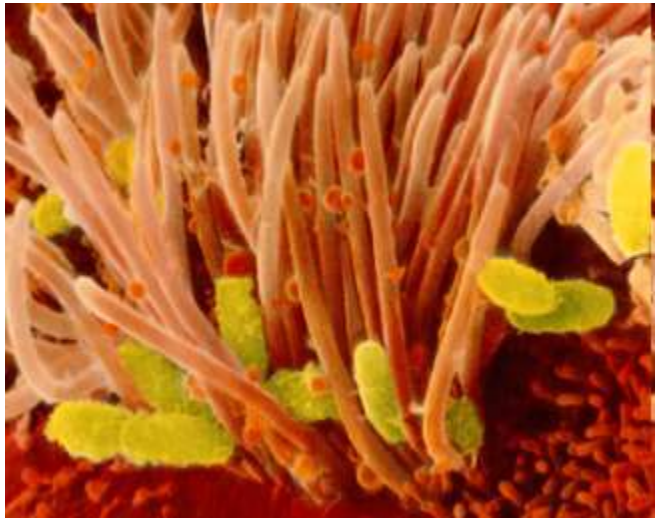
- aerobic, Gram-negative coccobacillus
- *Alcaligenaceae* family
- specific to humans
- whooping cough
- nutritionally fastidious – cultivated on media containing blood (source of many nutrients)
- 1906 – Bordet and Gengou



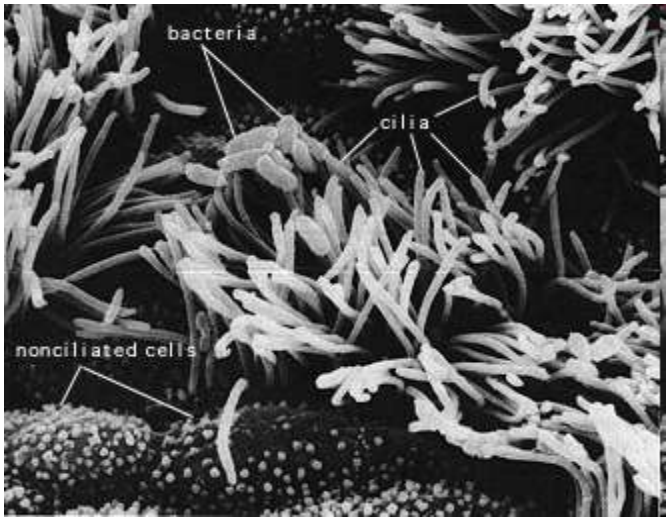
http://www.disease-picture.com

Bordetella Infection

- colonization of the respiratory tract
- biofilm formation (essential role of adhesins)
- binding to the ciliated epithelial cells in the nasopharynx and trachea and multiplication → death of the cells and/or loss of cilia, stop of cilliary beating → bacteria and mucus are not taken out of the airway → persistent coughing, but why so long if no bacteria around ?
- Persistence in the host due to intracellular survival?



http://children.webmd.com/



http://www.textbookofbacteriology.net

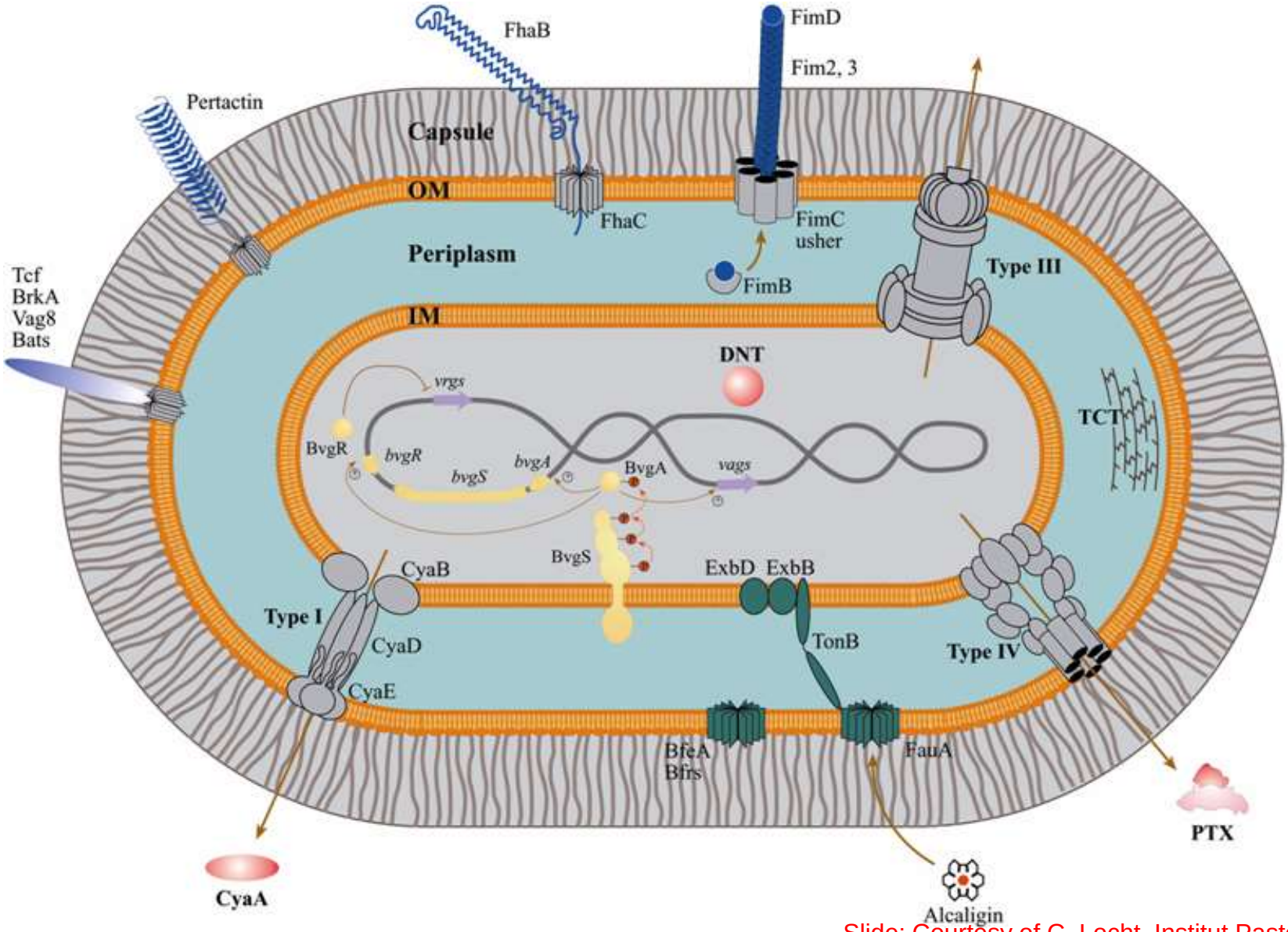
Despite medical importance and modern postgenomic tools, our understanding of pathophysiology of pertussis and of the real mechanisms of interaction with the human host is quite limited.

The reason is that until recently pertussis has been only been studied in the mouse intransal challenge model that does not reprodce human pertussis well enough.

Since 2010 a non-human primate model in baboon weanlings, reproducing human pertussis is available, BUT:

- Available in 2 labs in US only…
- A single small experiment costs ~$100,000 USD…

Bordetella pertussis is armed with numerous parallel virulence systems that mediate immune suppression



Slide: Courtesy of C. Locht, Institut Pasteur de Lille

B. pertussis Tohama I vaccine-type strain genome : 4,086,186 bp

Tohama I genome sequencing revealed on the top of what was known:

- **200 *IS***
- **130 transcription factors**
- **100 ABC transporters**
- **17 two-component syst.**
- **14 autotransporters**
- **Integrated phage**
- ***Hin*dIII restriction**
- **91 Bug (79 complete)**
- **FHA-like (FhaL, FhaS)**
- **Capsule synth. & export**
- **Intimin-like**
- **Flagellum (65 kb)**
- **Type III secretion syst.**
- **Exported proteases**
- **Siderophore/heme recept.**

How many of the transporters and other systems, of the vir-repressed genes, T3SS effectors and other gene products are involved in the intracellular life of *B. pertussis*???

B. pertussis adhesin and toxin confusion….

Adhesins

- **Filamentous haemagglutinin (FHA)**
- **Fimbriae**
- **Pertactin (RGD motif)**
- **Tracheal colonisation factor (TCF)**
- **Bps exopolysacccharide**

Serum resistance

BrkA, Vag8, capsule, LOS modification, C1inh binding

Toxins

- **Pertussis toxin (PTX)**
- **Adenylate cyclase (AC)**
- **Dermonecrotic toxin (DNT)**
- **Tracheal cytotoxin (TCT)**
- **Lipopolysaccharide (LOS)**

- Pertactin (neutrophil resistance)
- FHA – immunomodulation through IL-10

Whooping cough messages

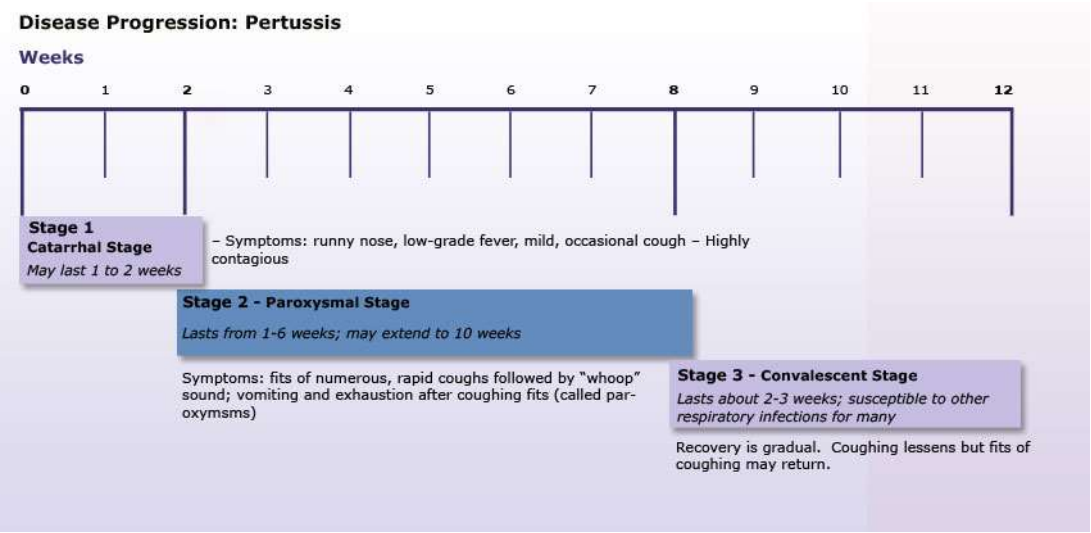
Disease

- Highly contagious
- Not inducing a life long immunity – **THE BACTERIUM SUPPRESSES IMMUNE MEMORY DEVELOPMENT** – can reinfect…
- Due mainly to *B. pertussis, B. parapertussis* and sometimes to *B. holmesii* and *B. bronchiseptica* in immunosuppressed subjects

Epidemiology

- Universal vaccination of young children induced a change in the transmission of the disease
- Adult and adolescent disease is increasing, and causes substantial disease burden
- Most infants are infected by an adolescent or adult contact

Whooping cough (pertussis)



– acute respiratory disease

– incubation period 4-21 days

– spread by aerosols or by direct contact with an infected person

– symptoms of heavy common cold (catarrhal phase with extremely runny nose and sneezing → followed by dry, paroxysmal phase cough (from weeks to months) and vomiting

– adults or immunized adults – any symptoms or milder form of the disease (up to 25 % with a persistent cough) → infection of nonvaccinated children

– treatment by erythromycin

– complications – pneumonia, encephalopathy

– Death of infants is often caused by secondary infections = superinfections by pneumococci or viruses that cause difficult to handle pneumonia

http://treatment-for.info/pertussis.html

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Audio/Video Products

Listen and watch to learn about pertussis symptoms, diagnosis, and prevention.

On this Page

Watch a short video of a boy coughing due to pertussis (http://streaming.cdc.gov/vod.php?id=7ffe0c683b0dc2765090991b8f8018c92012090410443264 7) (00:12 seconds)

Watch a short video of a hospitalized man with pertussis (http://www.nejm.org/action/showMediaPlayer?doi=10.1056/NEJMicm1111819&aid=NEJMicm1111819_attach_1&area) (http://www.cdc.gov/Other/disclaimer.html) (00:56 seconds)

(http://www.nejm.org/action/showMediaPlayer?doi=10.1056/NEJMicm1111819&aid=NEJMicm1111819_attach_1&area)

Hear how pertussis sounds in a child (http://www.pkids.org/dis_pert_stsop.php) (http://www.cdc.gov/Other/disclaimer.html)

(http://www.pkids.org/dis_pert_stsop.php)

http://www.cdc.gov/pertussis/pubs-tools/audio-video.html#pertussis-sounds

Problém s definicí případu

Definice případu

- Pro sjednocení případů v různých státech se používají jednotná kritéria, takzvané definice případu.
- Pod definicí případu se skrývají klinická, laboratorní a epidemiologická kritéria případu.
- Definice pertuse se v minulých letech měnila s nástupem dokonalejších a přesnějších laboratorních metod, přetrvávaly a dosud přetrvávají drobné rozdíly mezi definicemi případů v jednotlivých státech.

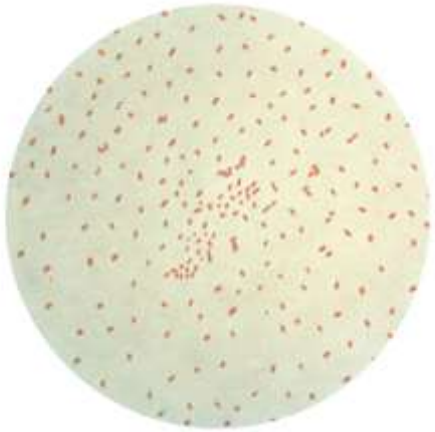
Definice případu se v americkém CDC vyvíjela následovně:

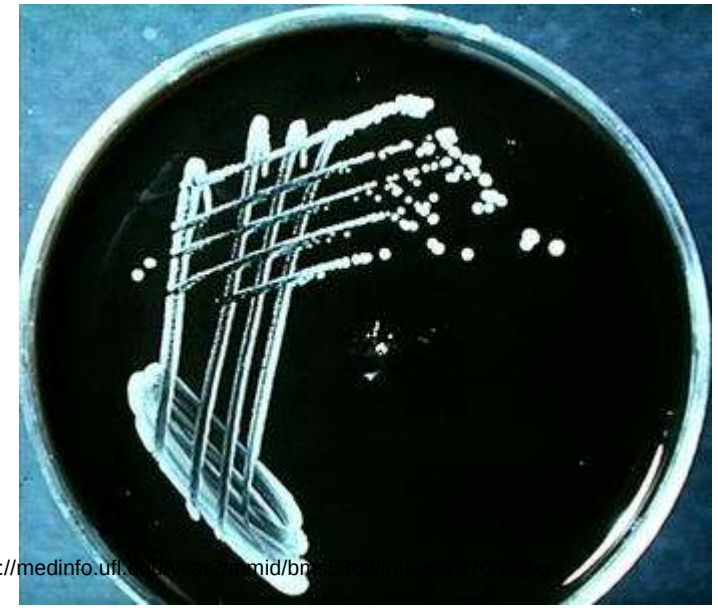
- Definice případů pertuse užívaná v letech 1990 – 1995 označovala případ za pravděpodobný pokud odpovídal klinické definici (kašel trvající déle než 2 týdny, doprovázeny paroxysmálním kašlem, zajíknutím nebo zvracením, a který je bez zřejmé příčiny) a zároveň nebyl laboratorně potvrzen a nebyl epidemiologicky spojen s nikym, u koho byla laboratorními testy prokázána infekce černým kašlem.
- Potvrzené jsou potom případy, kde se příznaky shodují s klinickou definicí (viz výše) a infekce je potvrzena buď laboratorními testy nebo je epidemiologicky spojena s někym, u koho byla laboratorními testy prokázána infekce. Tato definice oproti mladším definicím nevyužívá polymerázové řetězové reakce.
- Od 1. 1. 2014 platí odlišná definice případu pertuse. Klinicky je případ definován jako kašel trvající déle než 2 týdny doprovázen alespoň jedním z doprovodných symptomů (paroxysmální kašel, zajíknutí, zvracení, pro děti do 1 roku věku i apnoe). Laboratorní definice případu je úspěšná izolace bakterií *B. pertussis* z pacientova nosohltanu nebo pozitivní PCR na pertusi.
- Za pravděpodobný se případ považuje pokud splňuje klinickou definici s tím rozdílem, že může kašel trvat i kratší dobu než 2 týdny, a zároveň případ není potvrzen laboratorními testy nebo není epidemiologicky spojen s jiným laboratorně potvrzeným případem.
- Nově se odlišuje i definice pravděpodobného případu pro děti mladší 1 roku, která jej popisuje pozitivní klinickou definicí, potvrzenou buď pozitivní PCR na pertusi nebo kontaktem s laboratorně potvrzeným případem pertuse. Za potvrzený případ se považuje pacient s buď libovolně dlouho trvajícím kašlem a úspěšnou izolací bakterií *B. pertussis* z pacientova nosohltanu, nebo kašlem trvajícím déle než dva týdny, doprovázeny alespoň jedním z doprovodných symptomů (paroxysmální kašel, zajíknutí, zvracení, pro děti do 1 roku věku i apnoe) a je potvrzen pozitivní PCR na pertusi nebo kontaktem s laboratorně potvrzenym případem pertuse.
- Současná definice se neomezuje pouze na kašel trvající déle než dva týdny, ale na kašel trvající libovolně dlouho. Právě toto bylo dříve jednou z příčin poddiagnostikované pertuse.
- Definice případu z 90. let minulého století naprosto evidentně zkreslily výsledky studií acelulárních vakcín, ve kterých uměle zvýšily jejich účinnost.“

Diagnostic remains a nightmare…

Lot of unrecognized circulation of the bacterium and mild disease, assymptomatic immune carriers.

- isolation from nasopharynx
  - plating on selective media – Charcoal agar
- qRT-PCR
- serological testing often useless





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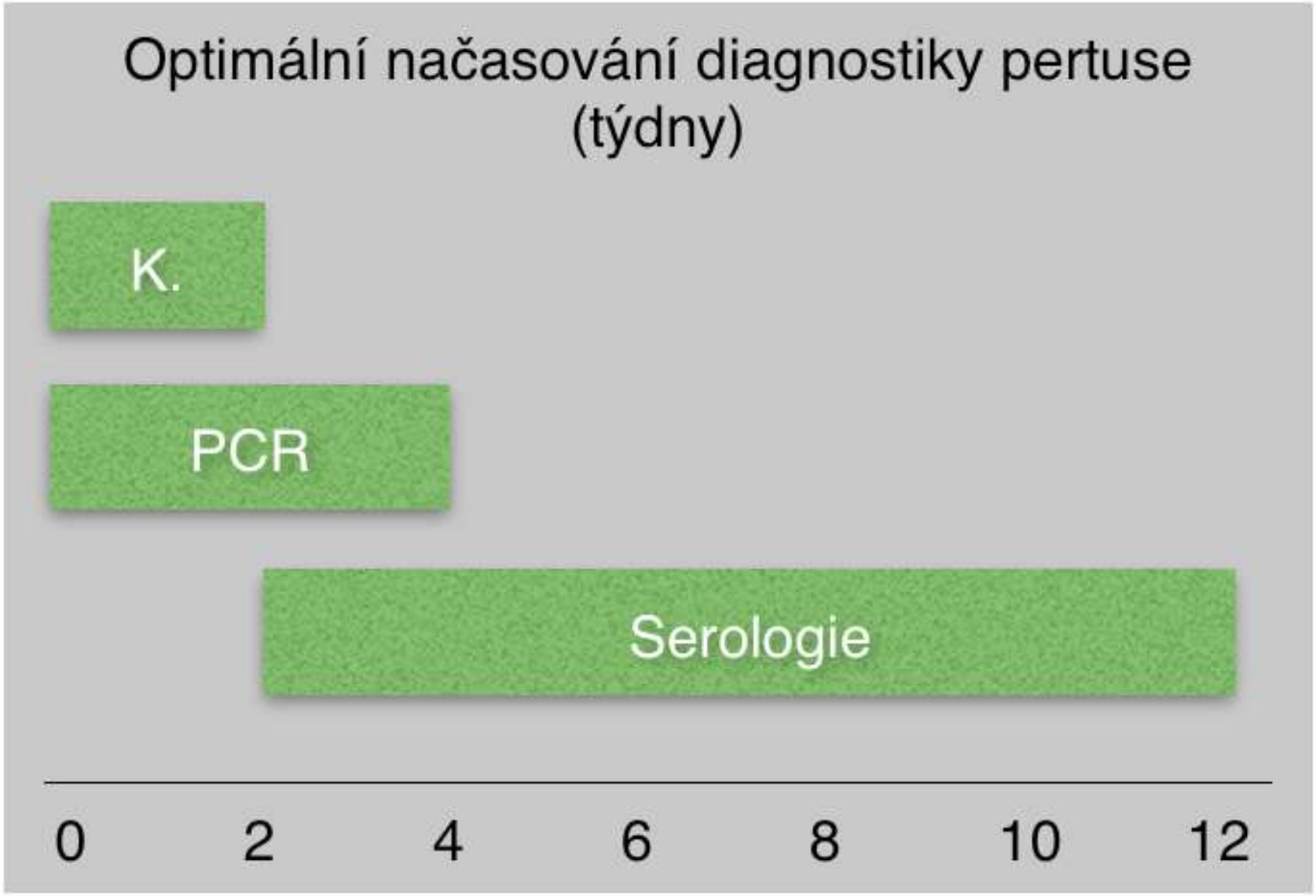
Optimální načasování diagnostiky pertuse
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Eur J Clin Microbiol Infect Dis (2011) 30:307–312
DOI 10.1007/s10096-010-1104-y

REVIEW

What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories

N. Guiso · G. Berbers · N. K. Fry · Q. He · M. Riffelmann · C. H. Wirsing von König · EU Pertstrain group

Received: 26 August 2010 / Accepted: 18 October 2010 / Published online: 11 November 2010

Abstract *Bordetella pertussis*-specific antibodies can be detected by enzyme-linked immunosorbent assays (ELISAs) or multiplex immunoassays. Assays use purified or mixed antigens, and only pertussis toxin (PT) is specific for *B. pertussis*. The interpretation of results can be based on dual-sample or single-sample serology using one or two cut-offs. The EU Pertstrain group recommends that: (i) ELISAs and multiplex immunoassays should use purified non-detoxified PT as an antigen, that they should have a broad linear range and that they should express results quantitatively in International Units per millilitre (IU/ml); (ii) a single or dual diagnostic cut-off for single-serum serology using IgG-anti-PT between 50 and 120 IU/ml should be used, and diagnostic serology cannot be validly interpreted for one year after vaccination with acellular pertussis (aP) vaccines; (iii) IgA-anti-PT should only be used with indeterminate IgG-anti-PT levels or when a second sample cannot be obtained. This group discourages using: (i) other antigens in routine diagnostics, as they are not specific; (ii) micro-agglutination, due to its lack of sensitivity; (iii) immunoblots for pertussis serodiagnosis, as results cannot be quantified; (iv) other methods, such as complement fixation or indirect immunofluorescence, due to their low sensitivity and/or specificity.

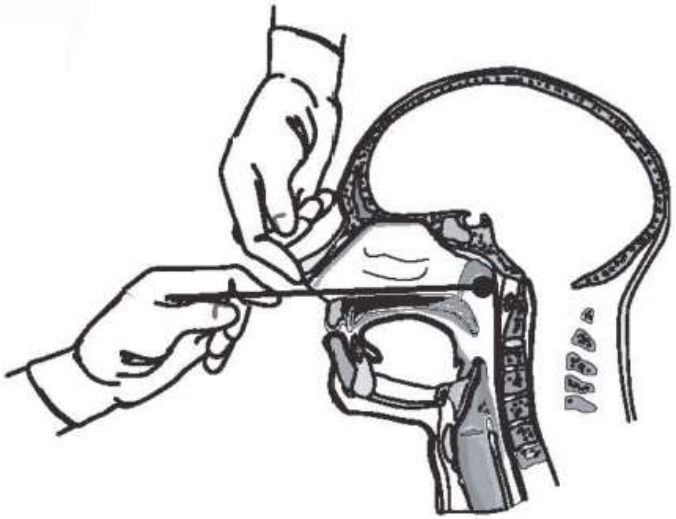
Indications for pertussis diagnostics

The diagnosis of pertussis should only be attempted in patients with symptoms compatible with pertussis, such as prolonged coughing with paroxysms and/or whooping or choking. In infants, older vaccinated children, adolescents and adults, the

Diagnostics method*	Relative sensitivity	Relative Specificity	Advantege(s)	Limitations
Clinical Diagnosis (symptoms)	+	++†		Low sensitivity in the vaccine era due to high proportion of atypical and mild pertussis cases
Culture‡	++	++++	Isolated strain may be subtyped	Varying sensitivity, depending on age and vaccination status
DFA	+	+	Rapid, non viable organisms also detected	Microscopist-dependent
PCR	+++	+++	More rapid than culture; increases the rate of case finding	Risk of false-positives; no standardized kits
Serology (ELISA)	+++	++++	Positive late in illness	Diagnosis possible only several weeks after onset; influenced by immunostatus; difficulty in correctly timing collection of samples paired sera; lack of inter-laboratory standardization of antibody titer cut-off (single-serum)

Comparison of diagnostics tests for detection and identification of *B. pertussis* * Zouari *et al.* (2012).
† Sensitivity and specificity for clinical diagnosis based on presence of cough for 2 or more weeks calculated during outbreak settings.
‡Varies in vaccinated versus unvaccinated populations, with age, prior antibiotic treatment, and also with duration of symptoms before sampling

Problémy s odběry



Obrázek 8: Nákres správně provedeného odběru vzorku z nasopharynxu

zdroj:
http://www.cdc.gov/vaccines/pubs/surv-manual/chpt10-pertussis.html

Pacient nesmí před odběrem jíst, kouřit, nesmí ani užívat antibiotika.

Pro odběr nesmí byt použit tampon z alginátu vápenatého, ktery zkresluje vysledky PCR, nybrž polyesterový tampon (např. Dacron nebo Rayon).

Vzorky jsou někdy odebírány z krku, což je chybné. Tampon má byt, jak ilustruje Obrázek 8, zaveden nosním průduchem až ke stěně pacientova nasopharyngu, kde má být ponechán několik sekund za současného otáčení a následně opatrně vyjmut.

Odběr je velice obtížný a vyžaduje trénink, proto je vhodné tímto úkonem pověřit ORL specialistu. Zákrok pro pacienta velice nepříjemný, u dětí je proto nutná fixace.

Tampon má byt po vyjmutí okamžitě ponořen do transportního média, kterým je AMIES s aktivním uhlím, a skladován má byt při pokojové teplotě, nikoliv v lednici.

Od odběru a zpracování vzorků smí uplynout maximálně 24 hodin.

Insertion sequence	Presence/ No. of copies per genome*			
	B. pertussis	B. parapertussis	B. holmesii	B. bronchiseptica†
IS481	+/ 238	-/ NA	+/ 8-10	(+)‡/ND
IS1001	-/ NA	+/ 22	-/ NA	(+)#/ 1-7
hIS1001	-/ NA	-/ NA	+/ 3-5	-/ NA
IS1002	+/ 6	+/ 90	-/ NA	-§/ 1

Bordetella insertion sequences

* +, present in all isolates; (+), present in some isolates; -, absent in all isolates; NA, not applicable; ND, not determined; Loeffelholz (2012).

† Human-derived *B. bronchiseptica* isolates only

‡ One of 73 human-derived isolates was positive

Four of 73 human-derived isolates were positive

§ Found in rare animal-derived isolates

Eur J Clin Microbiol Infect Dis (2013) 32:89–96
DOI 10.1007/s10096-012-1718-3

ARTICLE

Insertion sequences shared by *Bordetella* species and implications for the biological diagnosis of pertussis syndrome

A. Tizolova · N. Guiso · S. Guillot

Received: 22 June 2012 / Accepted: 27 July 2012 / Published online: 12 August 2012

Abstract The molecular diagnosis of pertussis and parapertussis syndromes is based on the detection of insertion sequences (IS) *481* and *1001*, respectively. However, these IS are also detected in the genomes of various *Bordetella* species, such that they are not specific for either *B. pertussis* or *B. parapertussis*. Therefore, we screened the genome of recently circulating isolates of *Bordetella* species to compare the prevalence of IS*481*, IS*1001* and, also IS*1002* with previously published data and to sequence all IS detected. We also investigated whether the numbers of IS*481* and IS*1001* copies vary in recently circulating isolates of the different *Bordetella* species. We used the polymerase chain reaction (PCR) method for screening the genome of circulating isolates and to prepare the fragments for sequencing. We used Southern blotting and quantitative real-time PCR for quantification of the numbers of IS. We found no significant diversity in the sequences of the IS harboured in the genomes of the *Bordetella* isolates screened, except for a 71-nucleotide deletion from IS*1002* in *B. bronchiseptica*. The IS copy numbers in the genome of recently circulating isolates were similar to those in reference strains. Our results confirm that biological diagnosis targeting the IS*481* and IS*1001* elements are not specific and detect the species *B. pertussis*, *B. holmesii* and *B. bronchiseptica* (IS*481*), and *B. parapertussis* and *B. bronchiseptica* (IS*1001*).

Introduction

We`ve got a real problem…

Whooping cough used to be a major cause of infant morbidity and death in the pre-vaccine era in CR

(slide dr. Fabianova NIPH)

Died of pertussis according to age groups in 1949-1957

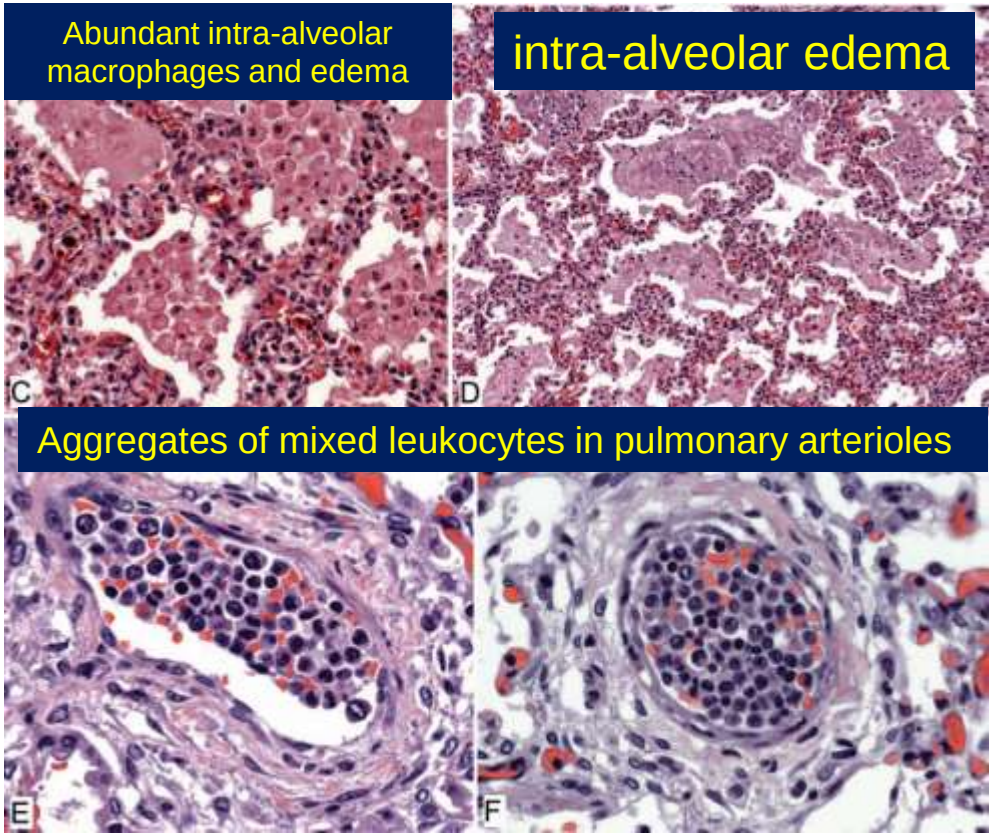
Procházka J., Kryl R. Praktický lékař, 6/1959

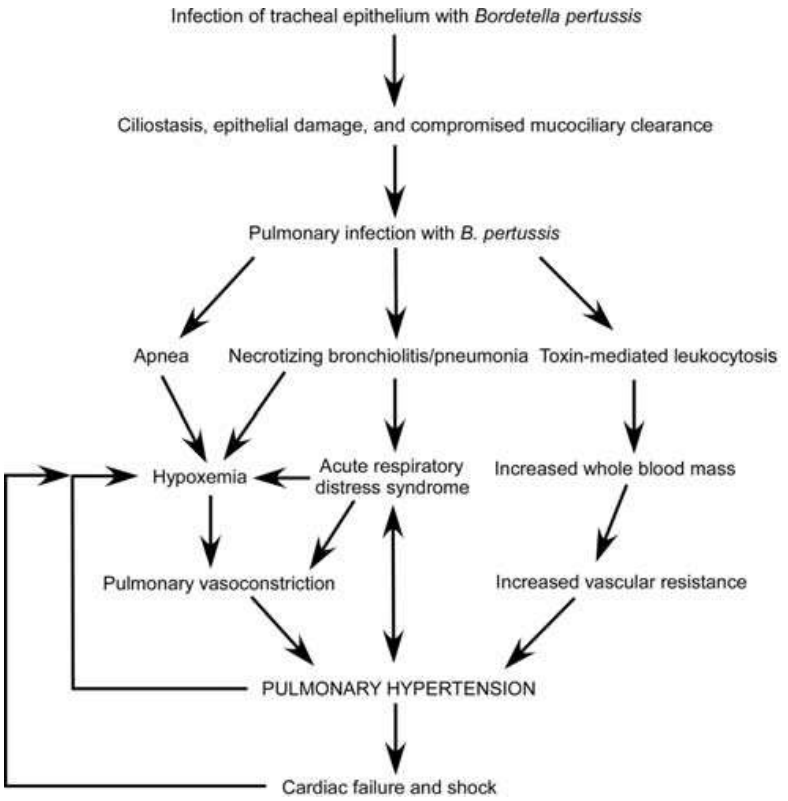
Věk v měsících	1949	1950	1951	1952	1953	1954	1955	1956	Celkem 1949-1956	Celkem %	1957	1957 %
- 1	673	280	463	234	276	157	89	87	2259	**76,0**	114	66,0
1-2	126	49	113	45	56	46	15	25	475	**16,0**	**96%!!!**	
2-3	30	11	16	12	13	21	11	9	123	**4,1**		
3-4	15	3	8	1	6	13	4	1	51	**1,7**		
4-5	3	0	6	2	4	5	1	1	22	**0,7**		
5-9	6	4	7	0	7	7	0	1	32	**1,1**		
10-14	2	1	0	0	0	1	0	1	5	**0,2**	59	34,0
15-19	0	0	0	0	0	0	0	0	0	**0,0**		
20-24	1	0	0	0	0	0	0	0	1	**0,0**		
25-29	0	0	0	1	0	0	0	0	1			
55-59	0	0	1	0	0	0	0	0	1	**0,2**		
Nezn. věk	0	0	2	0	0	0	0	0	2			
Celkem	856	348	616	295	362	250	120	125	2972	**100,0**	173	100,0

Paddock *et al.* Clinical Infectious Diseases 2008; 47:328–38

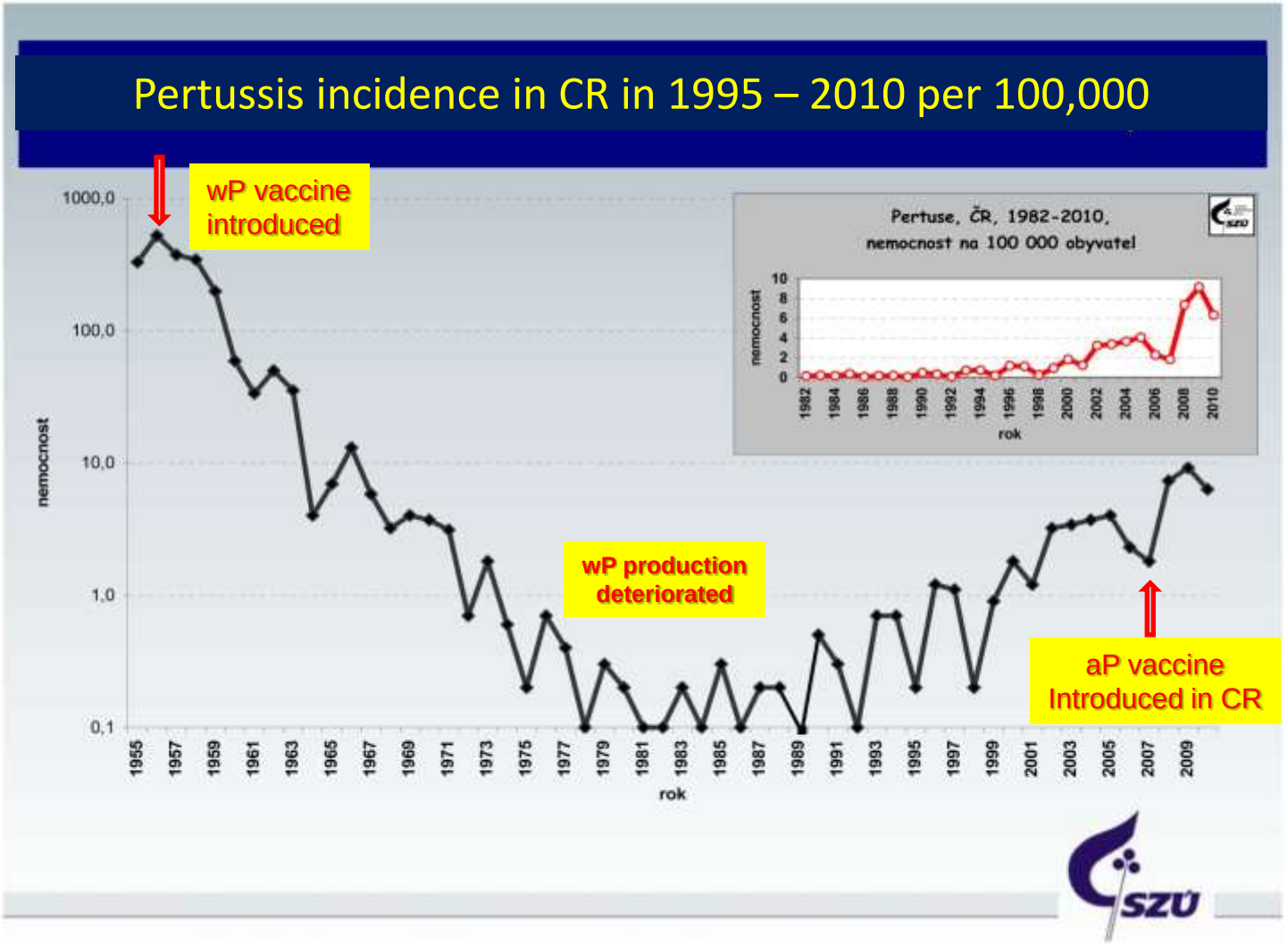
Pathology and Pathogenesis of Fatal *Bordetella pertussis* Infection in Infants

- Refractory pulmonary hypertension, leading to cardiac failure and shock, is now recognized as a frequent problem in infants with fatal pertussis

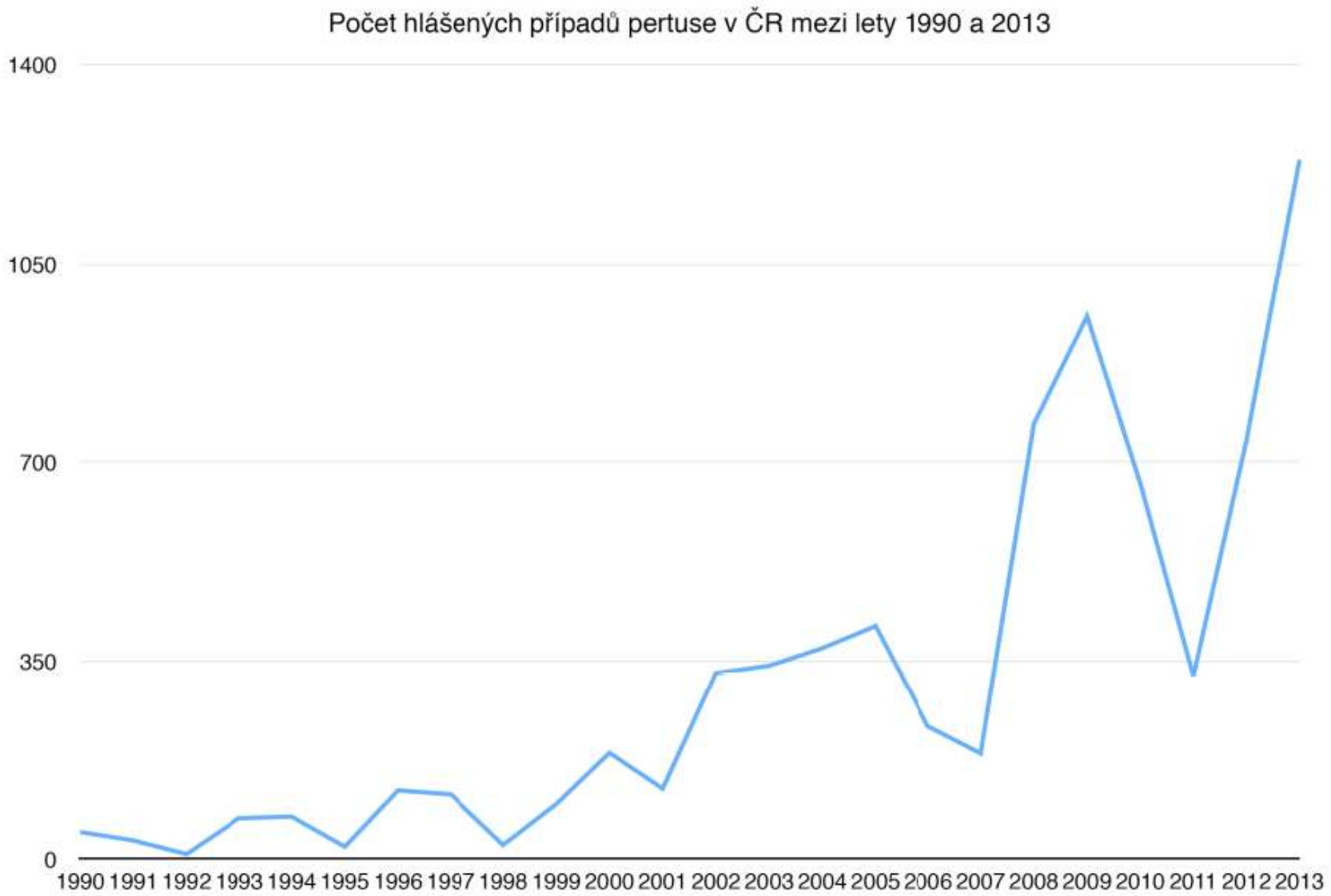




We thought the problem was solved...



Máme opět problém….



Obrázek 2: Graf vývoje incidence pertuse v CR mezi lety 1990 a 2013 (zdroj: SZU)

Antivakcinační kampaně mají fatální následky

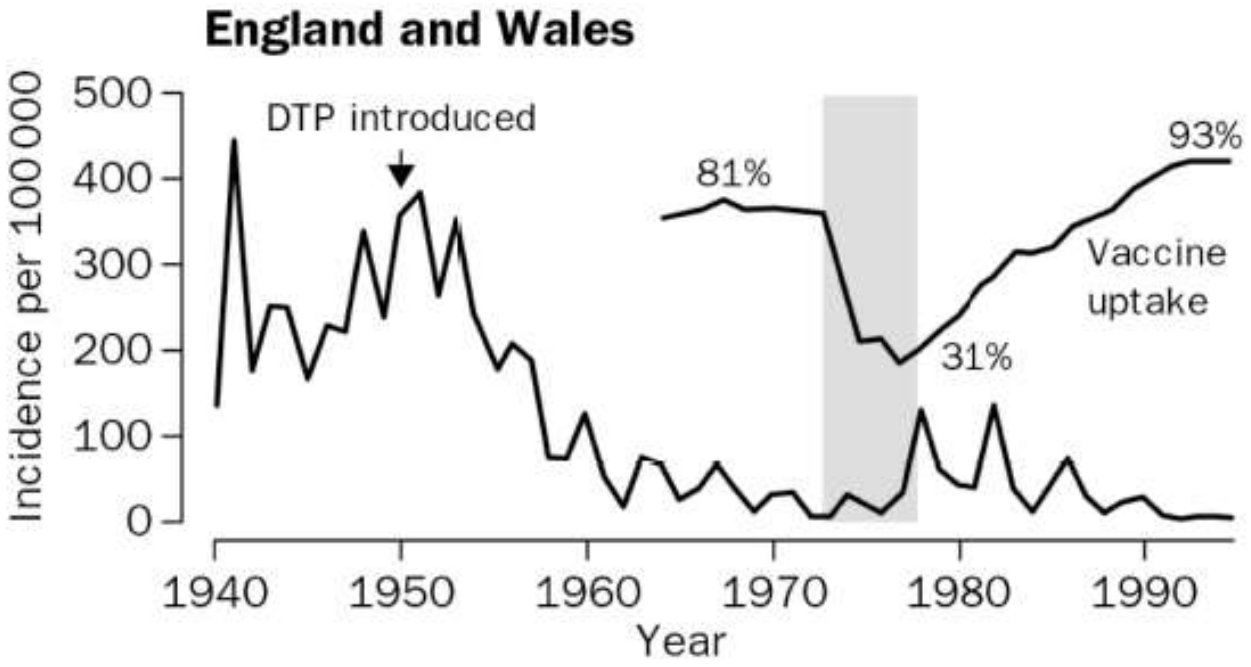


Figure 2: **Incidence of pertussis in countries affected by active anti-vaccine movements**

Obrázek 9: Graf vývoje incidence pertuse a proočkovanosti proti pertusi, Velká Británie

(zdroj: GANGAROSA E. J. et. al. *Impact of anti-vaccine movements on pertussis control: the untold story)*

Thanks to ‘democracy’ and introduction of acellular vaccines
the whooping cough is back to the most developed countries…

Are current pertussis acellular vaccines optimal?

PT only used in Denmark...

PT+FHA

PT+FHA + PRN

PT+ FHA +PRN+ FIM (2+3)

- There are issue on the:
  - Th1 *versus* Th polarity of induced immune response
  - Duration of B and T cell memory

- Several major *Bordetella* antigens are missing in the acellular vaccine:
  - LPS (LOS)
  - CyaA
  - T3SS (Bsp22) antigen – potent in *B. bronchiseptica model*
  - BrkA, no Tcf, and other of the adhesion/virulence factors

Isn`t it a miracle that detoxified PT only works as Pa vaccine???
B. parapertussis does not produce PT, causes whooping cough and is on the raise…

Název vakcíny	Výrobce	Typ	Obsažené antigeny (pertuse)	Licence
Daptacel	Sanofi Pasteur	DTaP	PT, FHA, PRN, FIM-2, FIM-3	všechny DTaP
Tripedia	Sanofi Pasteur	DTaP	PT, FHA	všechny DTaP
Infanrix	GlaxoSmithKline	DTaP	PT, FHA, PRN	všechny DTaP
Kinrix	GlaxoSmithKline	DTaP-IPV	PT, FHA, PRN	pouze 5. dávka a booster
Pediarix	GlaxoSmithKline	DTaP-HepB-IPV	PT, FHA	první 3 dávky, nemá licenci pro boostery
Pentacel	Sanofi Pasteur	DTaP-IPV/Hib	PT, FHA, PRN, FIM-2, FIM-3	první 4 dávky
Boostrix	GlaxoSmithKline	Tdap	PT, FHA, PRN	pro starší 10 let, booster
Adacel	Sanofi Pasteur	Tdap	PT, FHA, PRN, FIM-2, FIM-3	11 - 64 let, booster

Vysvětlivky:
DTaP — vakcína obsahující tetanový toxoid, záškrtový toxoid a některé antigeny pertuse
IPV — virus poliomyelitidy
HepB — virus hepatitidy B
Tdap — vakcína obsahující tetanový toxoid, redukovaný záškrtový toxoid a redukované antigeny pertuse
PT — pertusový toxoid
FHA — filamentosní hemagglutinin
PRN — pertactin
FIM-2 — fimbriae typu 2
FIM-3 — fimbriae typu 3

Obrázek 6: Seznam vakcín proti pertusi licencovaných v USA

2009-2012, Australia was facing a real whooping cough epidemic

up to 35,000 of cases in 2011…
despite quite good vacine coverage..

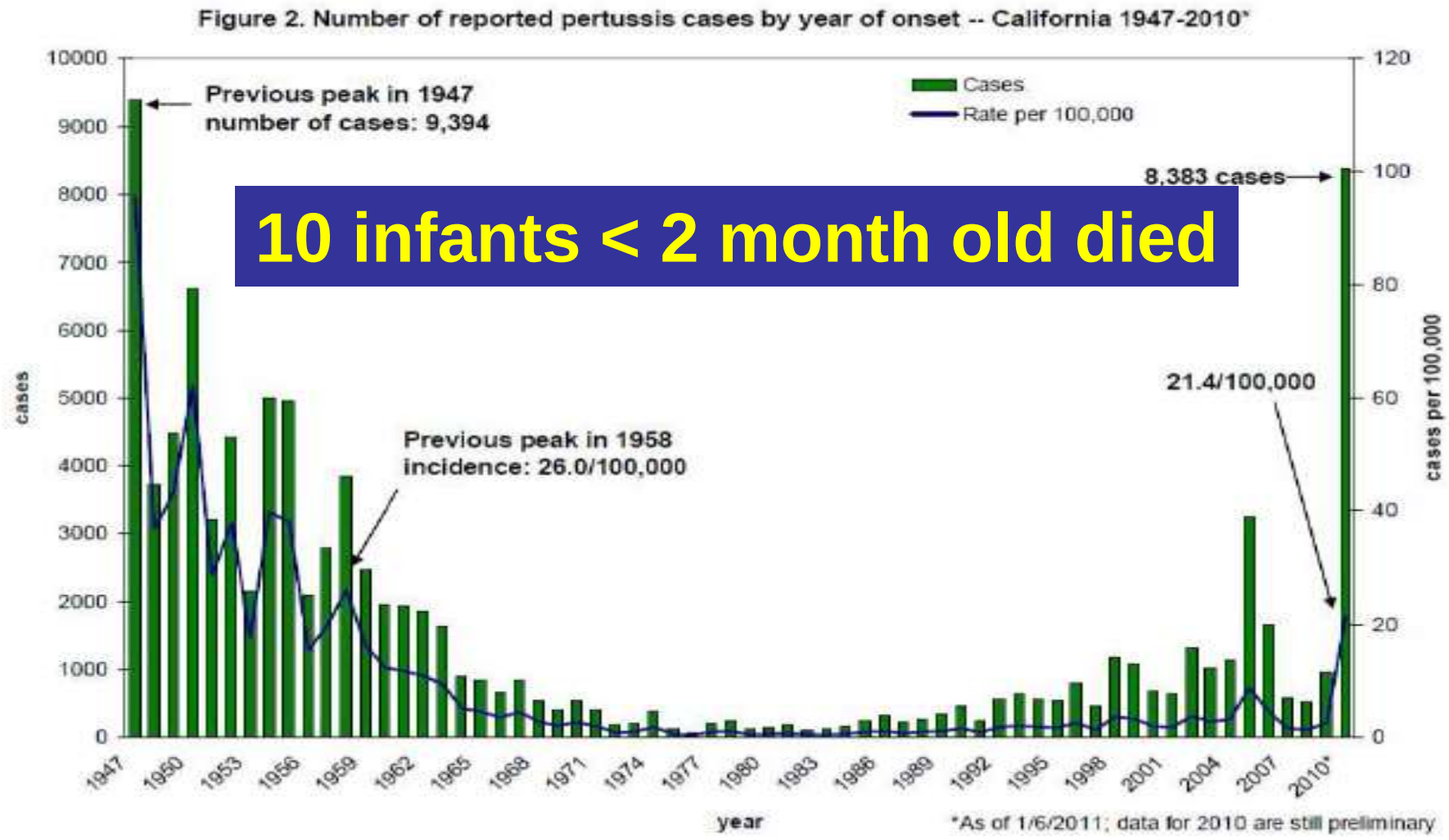
>100 cases/100,000 inhabitants…
(like in the pre/vaccine era….)

No booster at 2 years of age….
pertussis is spread by school children

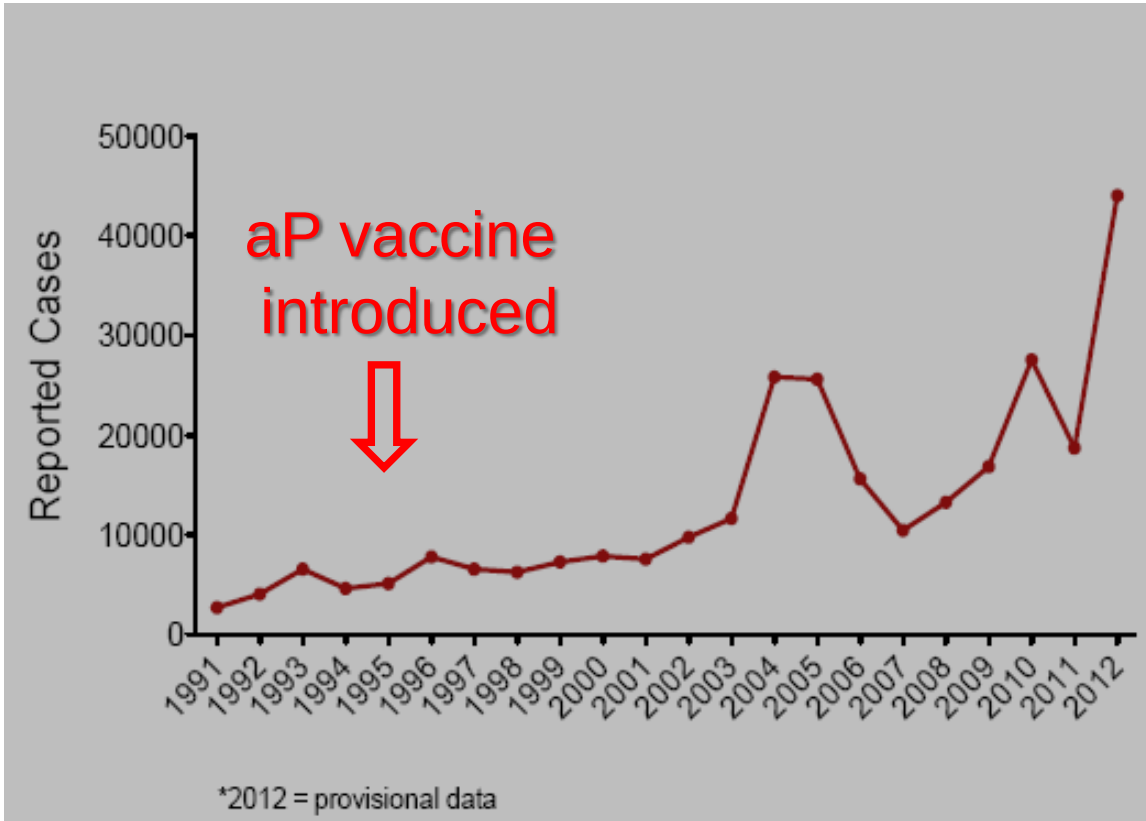
In 2012 the US had the highest whooping cough incidence since 70 years…
16 deaths

the 2010 California outbreak

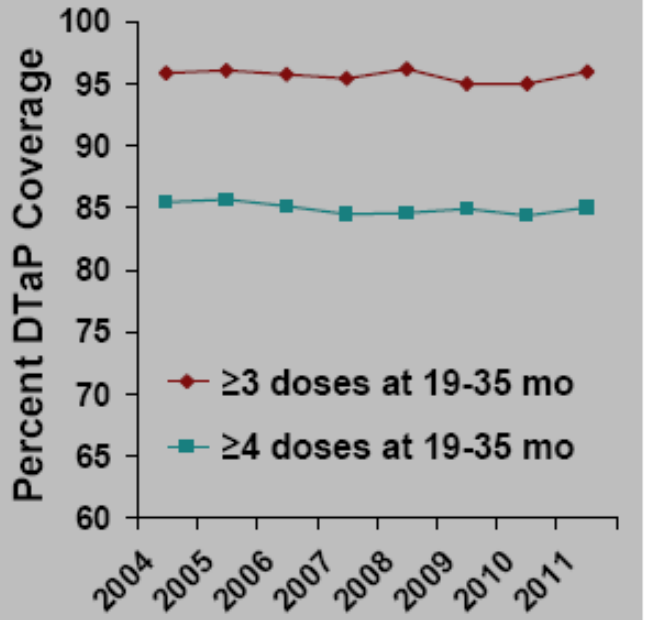
(we all wake up when something happens in the US…)



Pertussis is Resurging in the US Despite 96% Vaccine Coverage

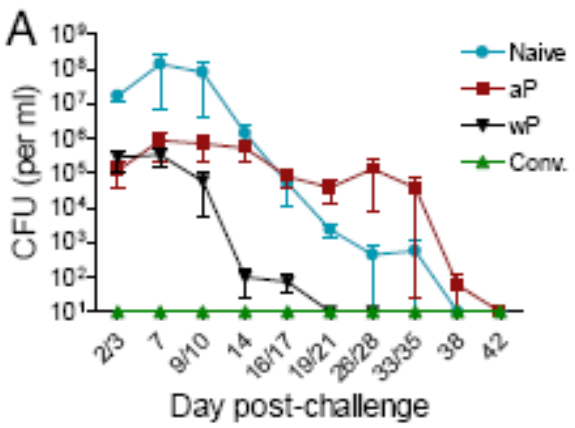


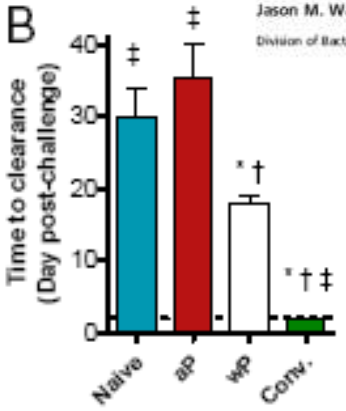
CDC National Notifiable Diseases Surveillance System

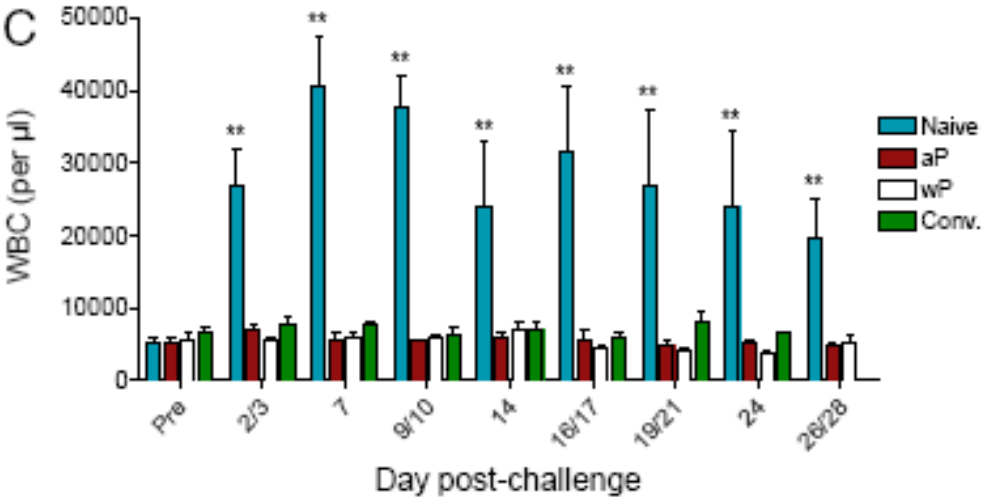


National Immunization Survey
Pertussis Surveillance System

aP vaccine protects against critical hyperleukocytosis and pneumonia but only wP protects against *B. pertussis* infection and transmission in humans (lot of literature) and in baboon weanlings







Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model

Jason M. Warfel, Lindsey I. Zimmerman, and Tod J. Merkel[1]

Division of Bacterial, Parasitic and Allergenic Products, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, MD, 20892

Warfel J M et al. PNAS 2014;111:787-792

www.pnas.org/cgi/doi/10.1073/pnas.1314688110

aP does not protect against *B. pertussis* colonization

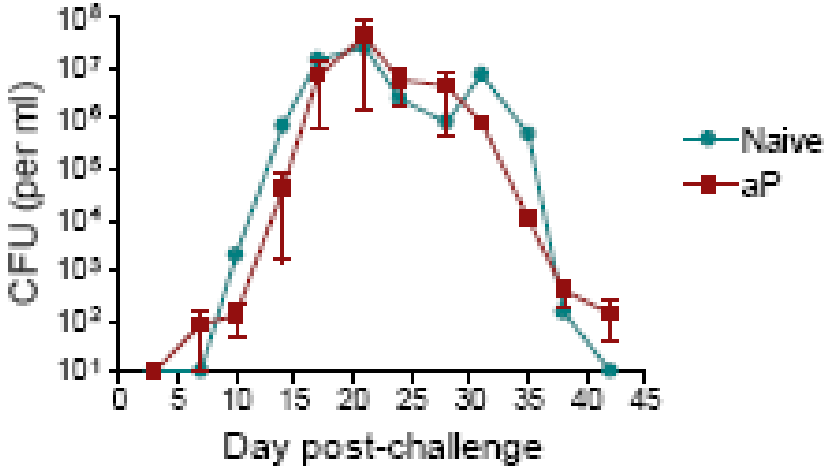


Fig. 2. aP does not protect against colonization following natural transmission. A naïve animal was directly challenged. After 24 h, a naïve animal and two aP-vaccinated animals were placed in the same cage as the directly challenged animal and followed for colonization as in Fig. 1.

Warfel J M et al. PNAS 2014;111:787-792

Infected aP vaccinees can transmit pertussis to naive contacts

Warfel J M et al. PNAS 2014;111:787-792

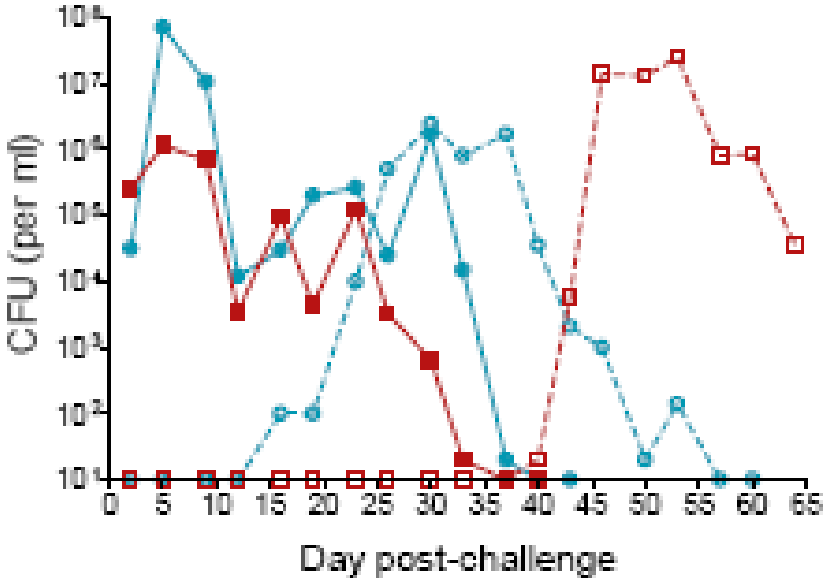
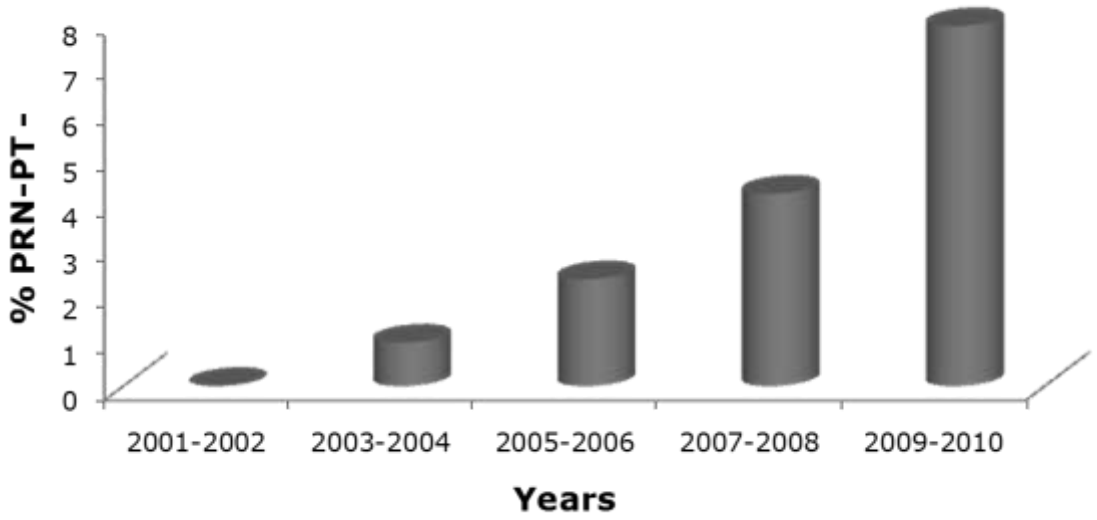


Fig. 3. Infected aP vaccinees can transmit pertussis to naïve contacts. Two animals vaccinated with aP were housed in separate cages, and each was directly challenged. Twenty four hours after challenge, an unchallenged naïve animal was placed in each cage. All animals were followed for colonization as in Fig. 1. One cage pairing is shown with turquoise lines with circles, and the other is shown with maroon lines with squares. Solid lines with closed symbols indicate the aP-vaccinated, directly challenged animals, and open symbols with dashed lines are used for the unchallenged, naïve contacts.

Bordetella pertussis – loss of aP antigens...

(slide courtesy of N. Guiso)

Species	Years									
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
B. pertussis	75	47	38	88	132	81	71	71	101	50
Vaccine Antigen not expressed	0/122		1PRN-/126		1PT-4PRN-/213		1PT-6PRN/142		12 PRN-1PRN-FHA-/151!	



Steady increase in the number of isolates non expressing an aP vaccine antigen

Similar findings in Japan

Bouchez et al, Vaccine, 2009 and In preparation ; N. Otsuka et al, poster, 2010

Pertactin-Negative Variants of *Bordetella pertussis* in the United States (2012)

Table 1. Characterization of *B. pertussis* Isolates from Philadelphia.*

Isolate	Date of Isolation	Patient Age	Pertactin Allele	Pertactin Mutation (Nucleotide)	Results of Western Blotting for Pertactin	PFGE Type
19–76	Jan. 2011	2 mo	*prn2*	None	Positive	CDC013
19–77	Feb. 2011	45 days	*prn2*	STOP (1273)	Negative	CDC002
19–81	March 2011	9 yr	*prn2*	IS (1613)	Negative	CDC237
20–2	May 2011	16 days	*prn2*	IS (1613)	Negative	CDC237
20–7	July 2011	40 days	*prn2*	STOP (1273)	Negative	CDC334
20–8	July 2011	78 days	*prn2*	STOP (1273)	Negative	CDC002
20–9	July 2011	83 days	*prn2*	STOP (1273)	Negative	CDC002
20–16	Sept. 2011	5 mo	*prn2*	STOP (1273)	Negative	CDC334
20–24	Oct. 2011	21 days	*prn2*	IS (1613)	Negative	CDC237
20–29	Feb. 2012	22 days	*prn2*	IS (245)	Negative	CDC010
20–30	Feb. 2012	11 days	*prn2*	STOP (1273)	Negative	CDC002
20–39	March 2012	14 yr	*prn2*	STOP (1273)	Negative	CDC002

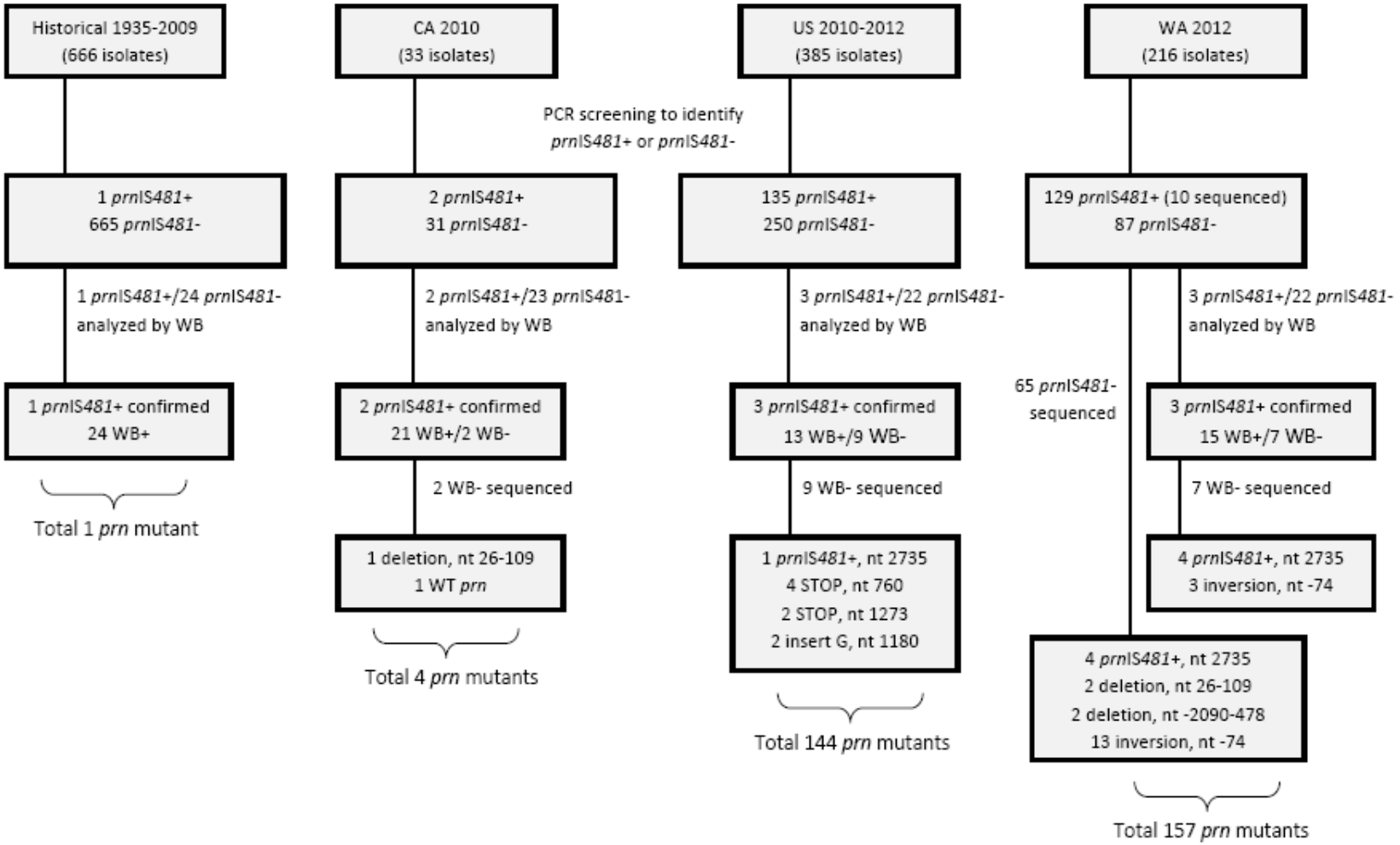
* IS denotes insertion sequence, PFGE pulsed-field gel electrophoresis, and STOP stop codon.

New England J. Med. 368:6 February 7, 2013

CVI Accepts, published online ahead of print on 20 November 2013
Clin. Vaccine Immunol. doi:10.1128/CVI.00717-13

Prevalence and molecular characterization of pertactin-deficient *Bordetella pertussis* in the US - > 50% of all recent isolates are PRN$^-$!!!

FIG 1.



So, does pertussis resemble this?

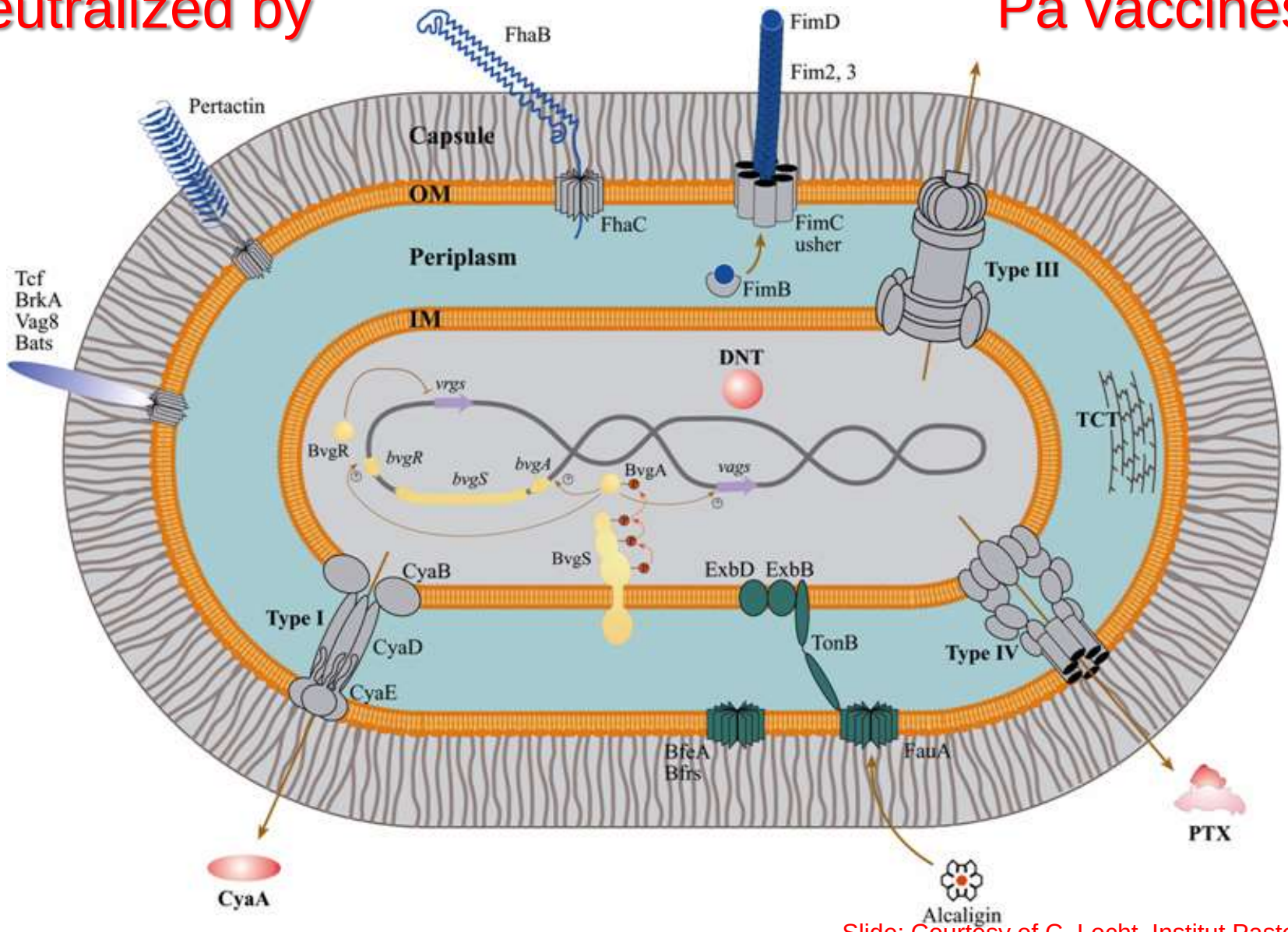
We hope it does not, but....

We need a much deeper understanding
of *B. pertussis* biology and virulence
very different pathology in kids and in mice….

We urgently need to develop much more efficient vaccines to prevent colonization and spread….

or should we develop next generation of non-reactogenic wP vccines…? – YES!!!

Bordetella pertussis is armed with numerous parallel virulence systems (cytotoxins, immunomodulators and adhesins) that are not neutralized by Pa vaccines



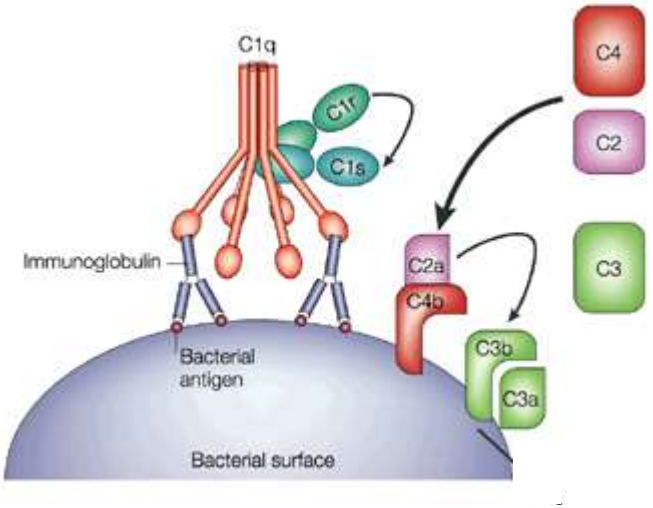
Slide: Courtesy of C. Locht, Institut Pasteur de Lille

Complement resistance

Complement system

- inhibition of complement-induced phagocytosis - **BrkA (*Bordetella* resistence to killing A)**

→ interference with the classical pathway of complement activation



http://www.nature.com/nri/journal/v2/n5/fig_tab/nri800_F1.html

- interfers with the deposition of C4b, C2a, and C3b → preventing phagocytosis and killing by neutrophils
- binds and recruits the major inhibitors (C4b-binding protein and human C1 esterase inhibitor)

Molecular Microbiology (2010) 77(6), 1439–1455

The Bps polysaccharide of Bordetella pertussis promotes colonization and biofilm formation in the nose by functioning as an adhesin

data reveal a biofilm lifestyle for *B. pertussis* in the nose and the requirement of Bps in this developmental process.
Bps functions as an adhesin by promoting adherence of *B. pertussis and Escherichia coli* to human nasal but not lung epithelia.

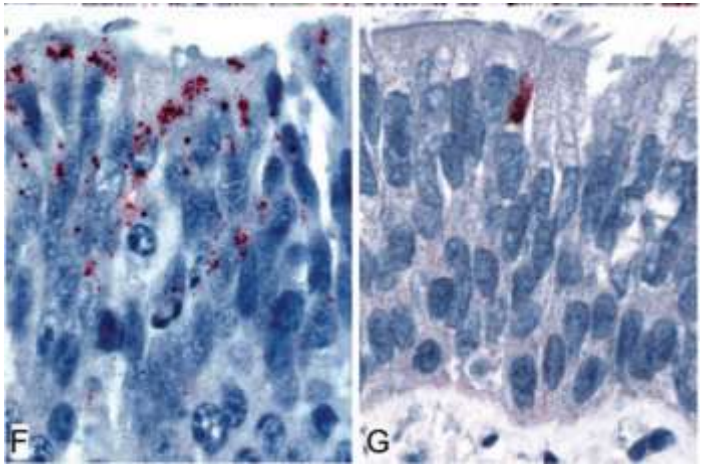
Cell Microbiol. 2014 Jan 20. doi: 10.1111/cmi.12264.
The Bordetella pertussis Bps polysaccharide enhances lung colonization by conferring protection from complement-mediated killing.
Ganguly T *et al.*

Is there an intracellular life of *B. pertussis?*

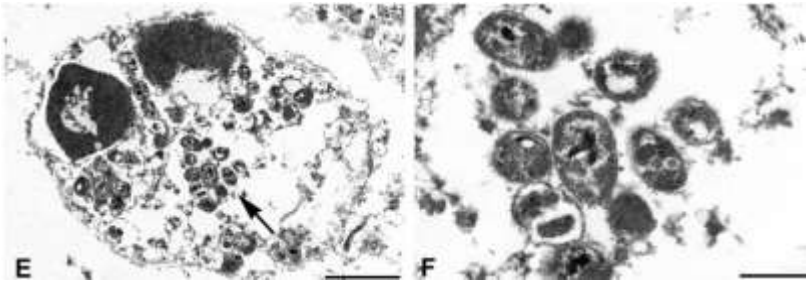
Paddock *et al*. Pathology and Pathogenesis of Fatal Bordetella pertussis Infection in Infants. Clinical Infectious Diseases 2008; 47:328–38

- **Animal models** of pertussis and in vitro studies using **human monocytes**
- ***B. pertussis* can enter, survive, and persist in macrophages for 40 days** contributing significantly to the total bacterial load in the lungs
- **in the early 1930s, *B. pertussis* was routinely found by culture of lung specimens from patients who died within 30 days after illness onset…**
- Bacterial residence within macrophages may allow survival of antibotic treatments

  Persistence of bacterial antigens in airway epithelium may also contribute to the chronicity that characterizes the clinical syndrome of pertussis.
- Bordetellae were identified in the cytoplasm of ciliated columnar epithelial cells, consistent with the findings from studies that have reported invasion of mouse ependymal cells and cultured human tracheal epithelial cells with B. pertussis.
- ***Bordetella* antigens were stained in the cytoplasm of tracheal epithelial cells from the upper respiratory tract of a died kid several weeks after antibiotic therapy, suggesting that bacterial antigens may persist in for at least** .



F, Intracellular *Bordetellae* and bacterial antigens in the columnar epithelium of a bronchiole *G, Bordetella* antigens in the cytoplasm of a tracheal epithelial cell in an infant given treatment for pertussis (**68 days after onset of symptoms and 57 days after isolation of *B. pertussis by culture*.)**



Intact coccobacilli *(arrows) fill the cytoplasm of a pulmonary macrophage (E and F).*

Is there an intracellular life of *B. pertussis?*

INFECTION AND IMMUNITY, Mar. 2010, p. 907–913 Vol. 78, No. 3
0019-9567/10/$12.00 doi:10.1128/IAI.01031-09

Intracellular Trafficking of *Bordetella pertussis* in Human Macrophages[▽]

Yanina A. Lamberti,[1] Jimena Alvarez Hayes,[1] Maria L. Perez Vidakovics,[1]†
Eric T. Harvill,[2] and Maria Eugenia Rodriguez[1]*

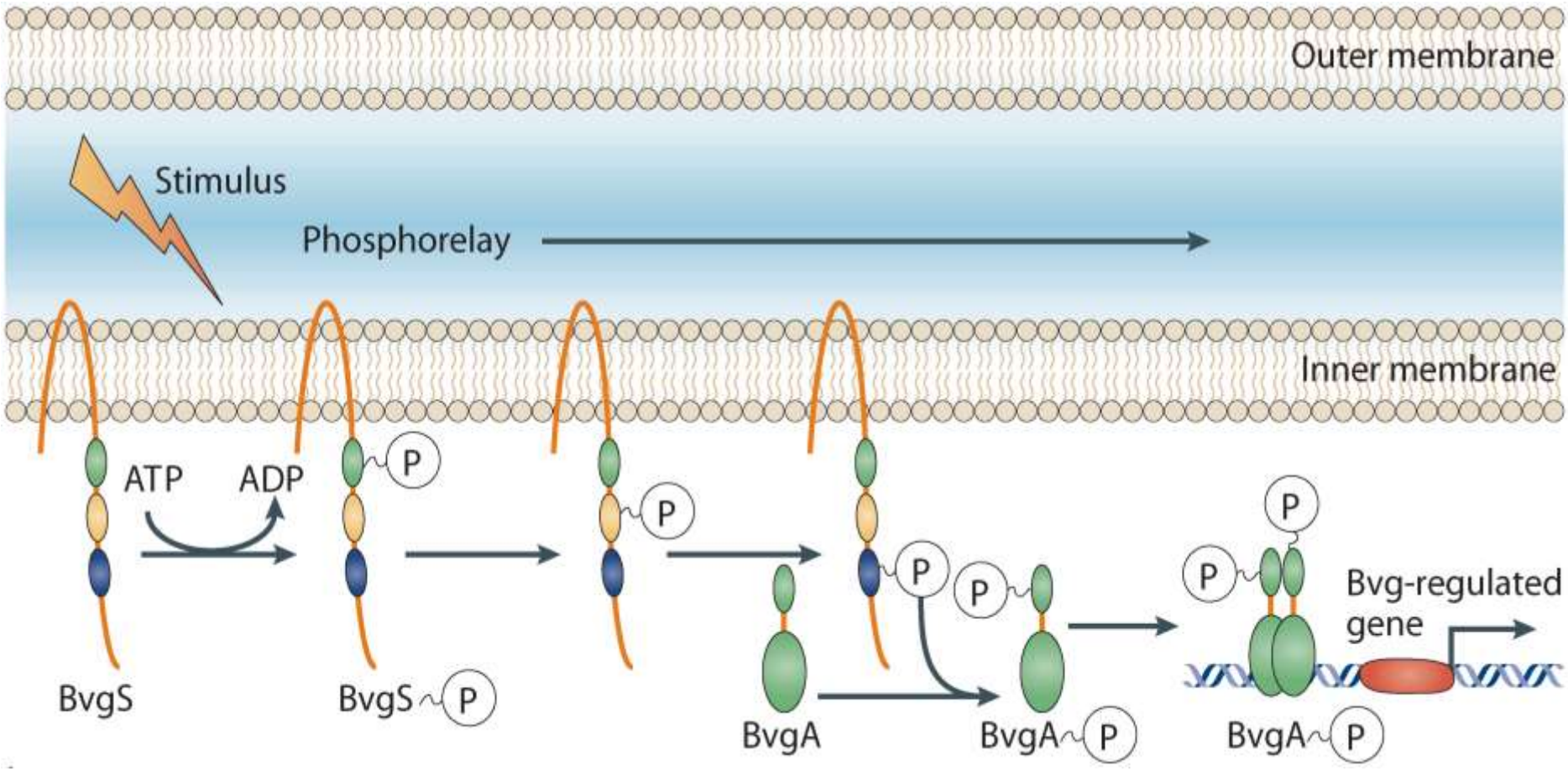
CINDEFI (UNLP CONICET La Plata), School of Science, La Plata University, La Plata, Argentina,[1] and Department of Veterinary and Biomedical Science, The Pennsylvania State University, University Park, Pennsylvania[2]

Received 9 September 2009/Returned for modification 10 September 2009/Accepted 31 December 2009

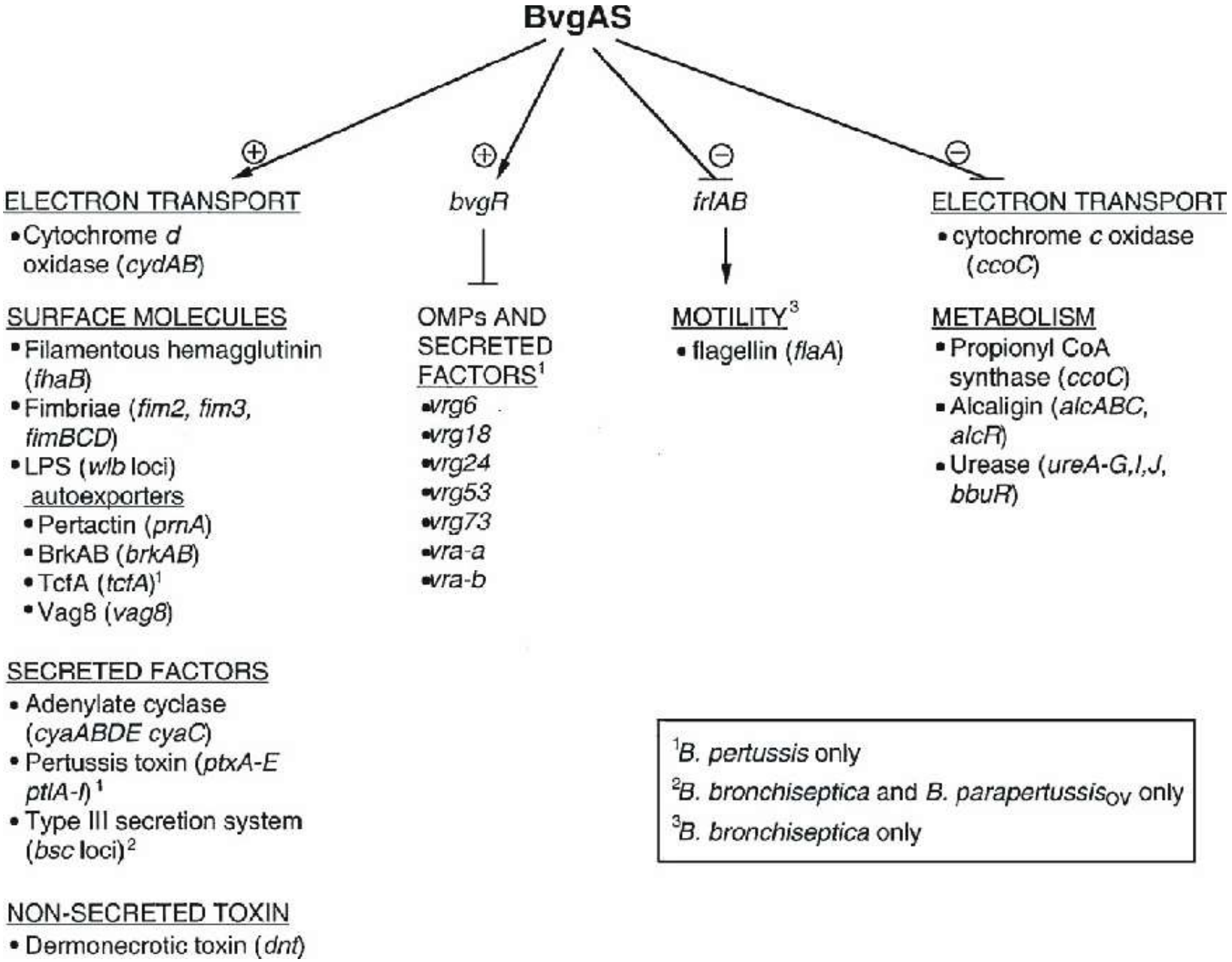
- During the first hours following phagocytosis, a high percentage of bacteria were destroyed
- roughly **one-fourth of the bacteria evade initial killing in nonacidic compartments.**
- At 48 h after infection, the number of intracellular bacteria per cell increased = grow
- Viable bacteria accumulated within phagosomal compartments positive for Rab5
- *B. pertussis*-containing phagosomes acquired exogenously added transferrin = intracellular bacteria have access to extracellular components and essential nutrients
- Contribution to persitence in hosts and populations?

BvgAS regulatory system

modulating conditions (sulfate or nicotinic acid or growth temperature below 25°C) → BvgAS phosphorelay is inactivated → no expression of virulence genes → avirulent Bvg⁻ phase



The Bvg-regulon of *Bordetella* species



Bordetella pertussis *in vitro* phenotypic modulation

37 °C

C mode ⇌ **X mode**

25°C
$MgSO_4$
Nicotinic acid

Vrgs

Vags

PTX
FHA
AC
Pertactin
T3SS

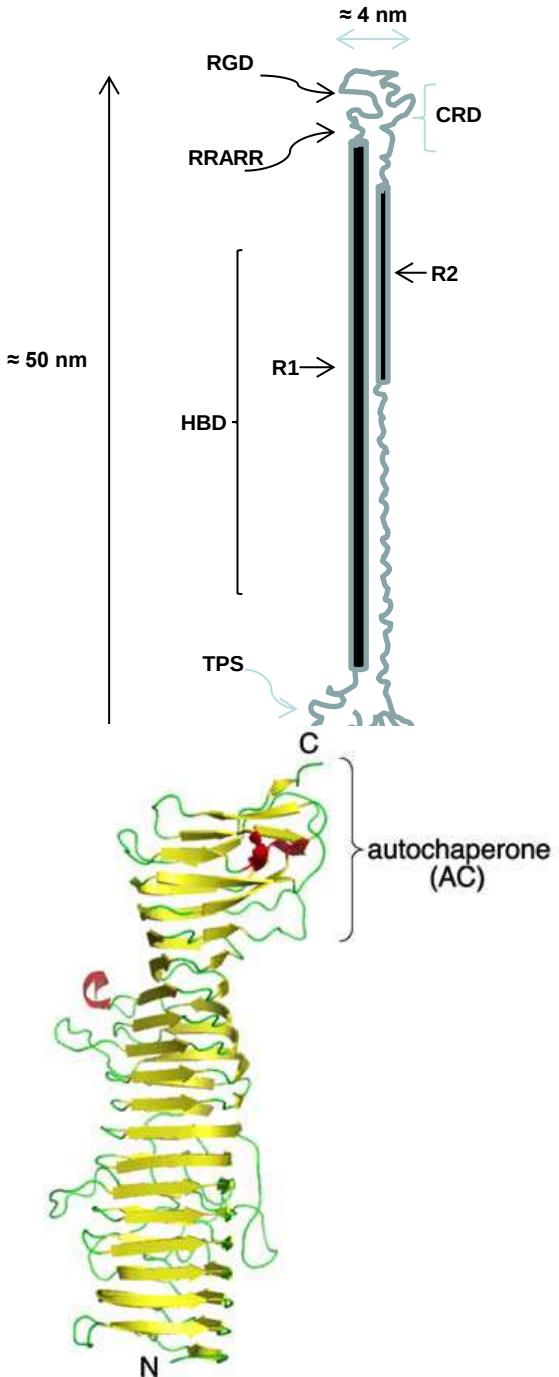
What does BvgAS sense in vivo????
Is it lower temperature in nasopharynx prior transmission?

Adhesins

Filamentous hemagglutinin (FHA)

- mature FHA noncovalently bound to the cell surface weaker interaction between bacteria and host
- binds galactose residues on sulfated glycolipids (ciliated cells) and CD11b/CD18 complement receptor (neutrophils)

- **Induces immunosuppressive IL-10 …. More of a toxin than an adhesin?**



Fimbriae

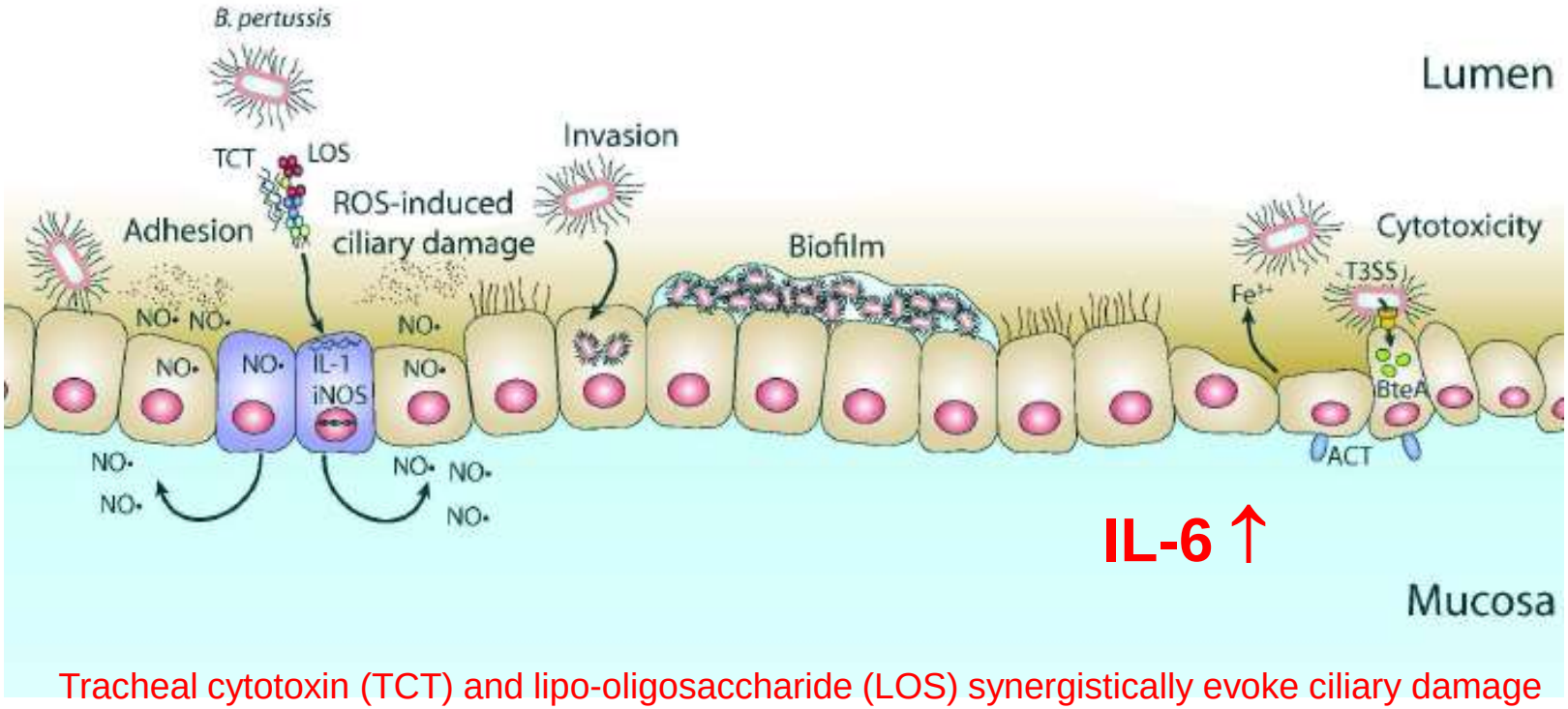
Pertactin (PRN) – involved in resistance to neutrophile clearance

What about the role of the other 11 autotransporters….????

Toxins

the Yang:

Pathologic effects of *Bordetella* toxins on respiratory mucosa…



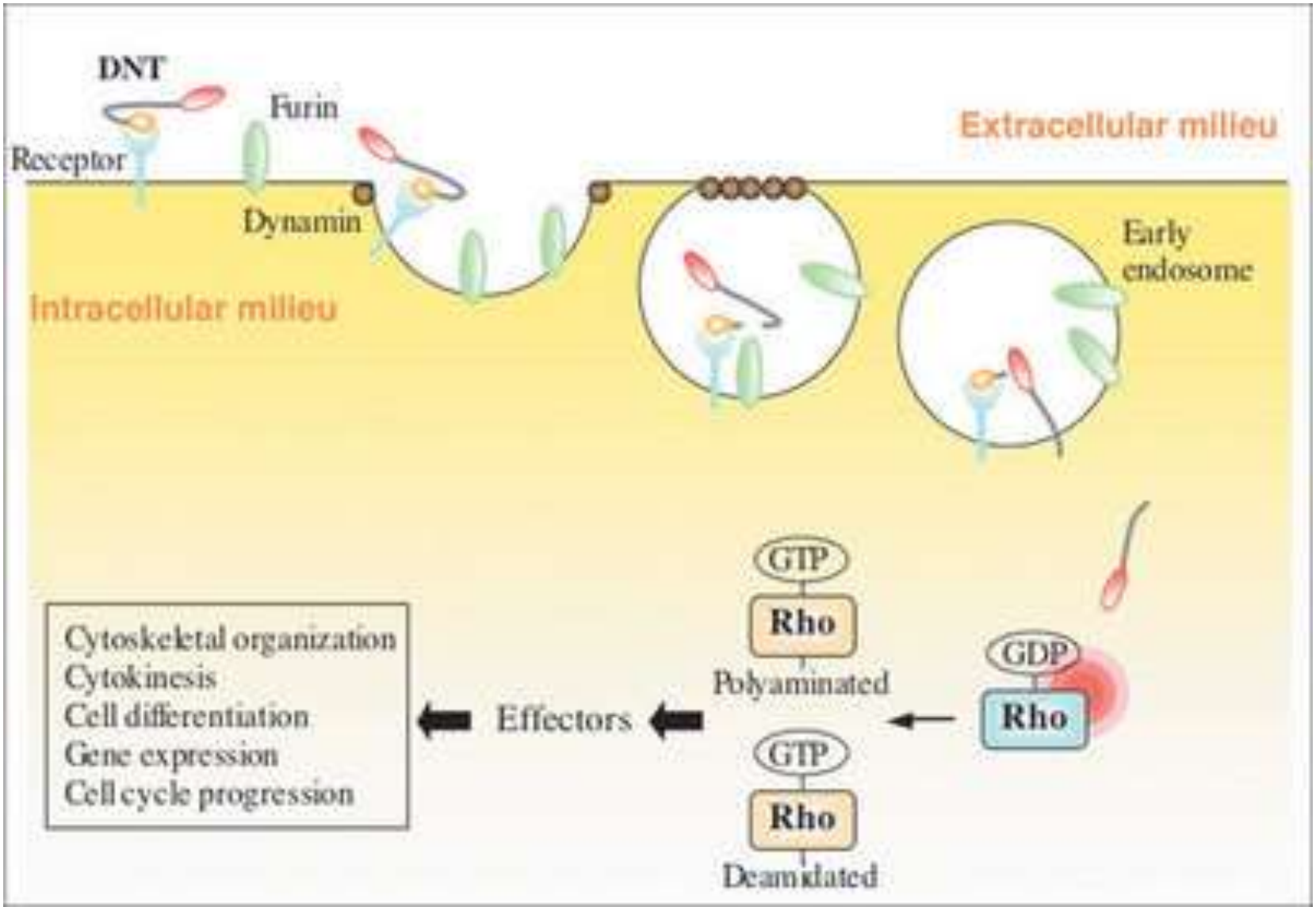
Tracheal cytotoxin (TCT) and lipo-oligosaccharide (LOS) synergistically evoke ciliary damage by initiating the release of destructive reactive oxygen species (ROS), such as nitric oxide (NO) via interleukin 1 (IL-1) induced type II nitric oxide synthases (inducible NOS, or iNOS) activation in mucus-secreting goblet cells

Adenylate cyclase toxin (ACT) and the type III secretion system (T3SS) with its effector protein BteA subvert intraepithelial signaling pathways leading to cytotoxicity.

COX2 activation and PGE_2, cytokines and chemoattractants secretion
Mucus production and lack of ciliary beating provokes COUGH !!!

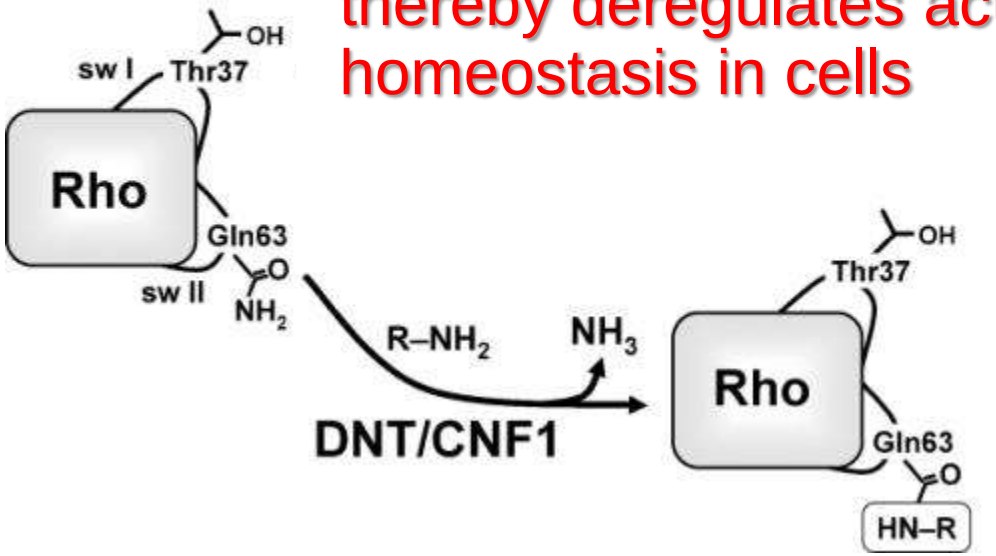
de Gouw *et al.* (2011) FEMS Microbiol. Rev. **35,** 441-474

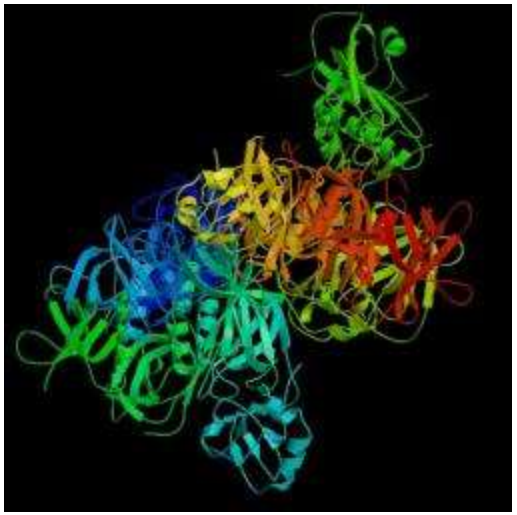
Dermonecrotic toxin is released by lysis of bacteria and causes deregulation of actin cytoskeleton homeostasis

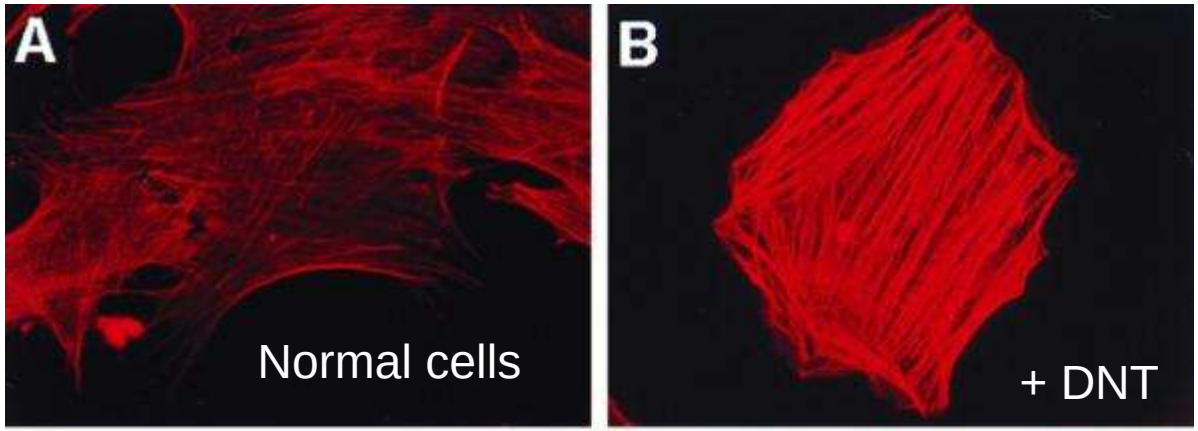


Bordetella Dermonecrotic toxin action

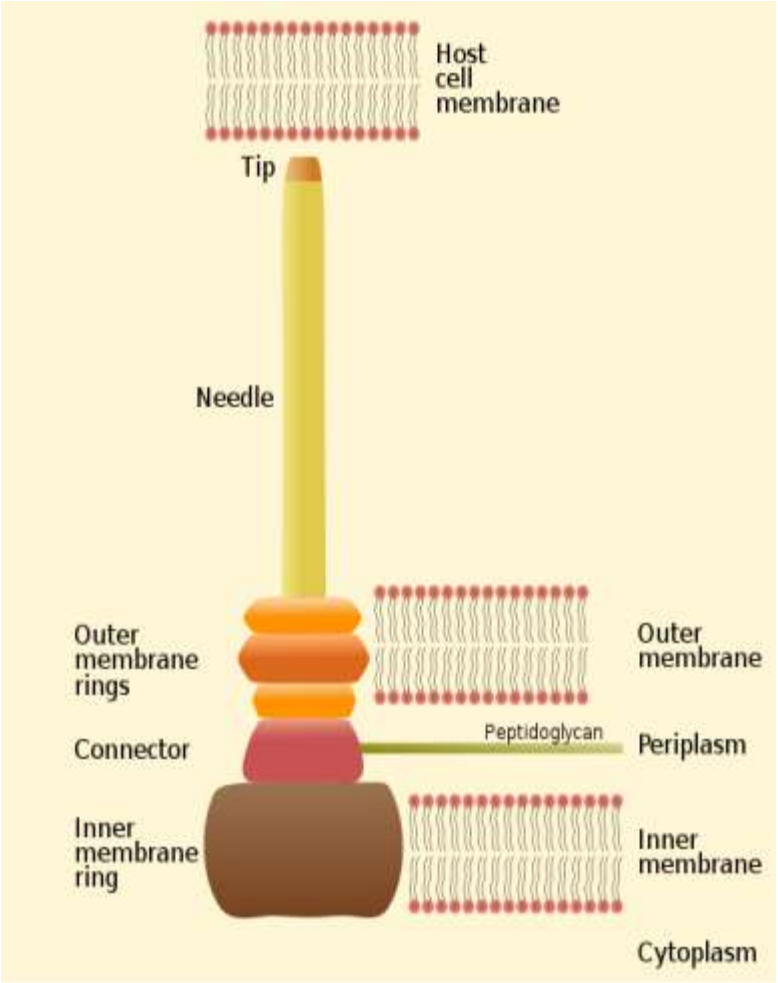
Causes deamination of Glutamine 63 of the small GTP-ase Rho and thereby deregulates actin homeostasis in cells

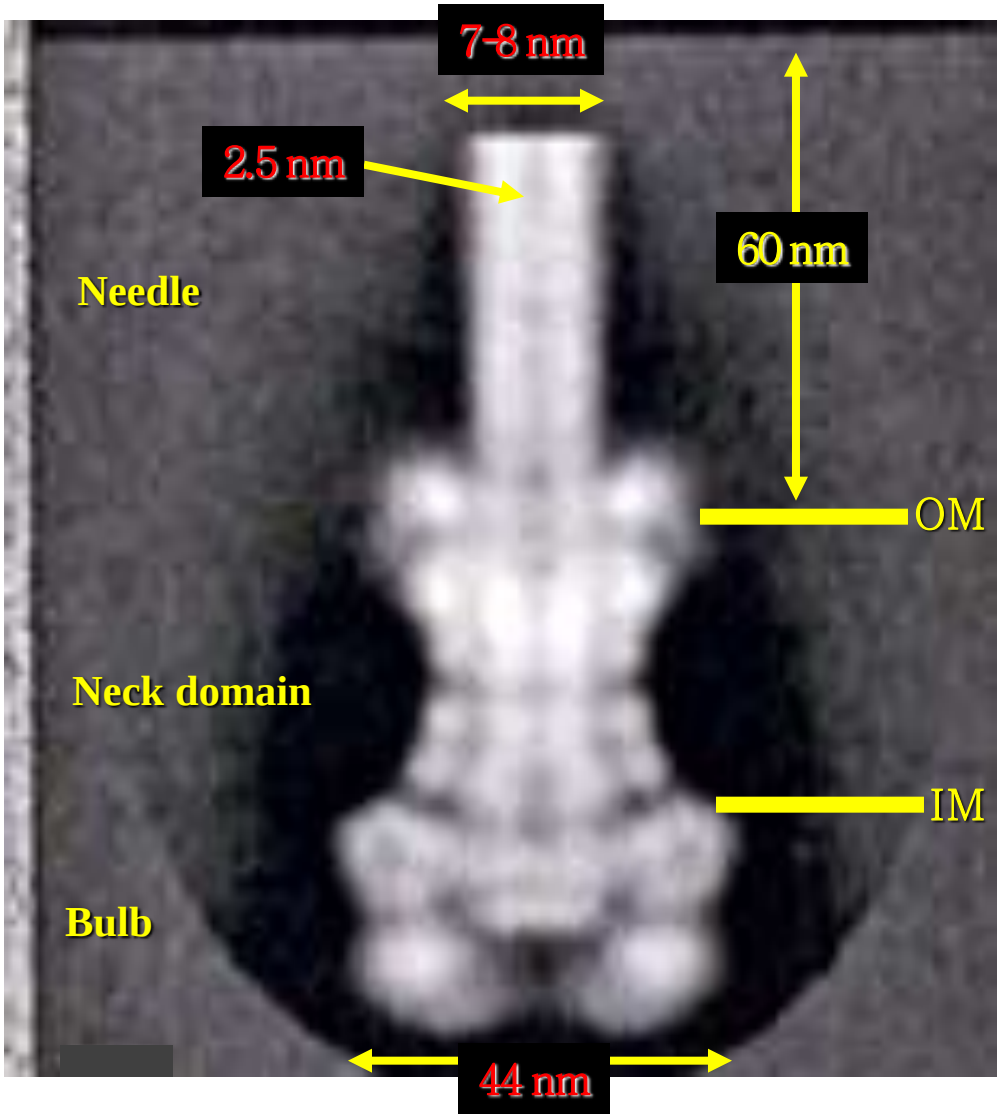






Bordetella evades the host immune system by Inducing IL-10 through a type III effector, BopN???





INFECTION AND IMMUNITY, Sept. 2011, p. 3677–3682 Vol. 79, No. 9
0019-9567/11/$12.00 doi:10.1128/IAI.00136-11

Laboratory Adaptation of *Bordetella pertussis* Is Associated with the Loss of Type Three Secretion System Functionality[▽]

M. E. Gaillard, D. Bottero, C. E. Castuma, L. A. Basile, and D. Hozbor*

Instituto de Biotecnología y Biología Molecular, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata CONICET, Calles 47 y 115, La Plata 1900, Argentina

Received 10 February 2011/Returned for modification 15 March 2011/Accepted 21 June 2011

Although *Bordetella pertussis* contains and transcribes loci encoding type III secretion system (TTSS) homologues, expression of TTSS-associated proteins has been reported only for non-laboratory-adapted Irish clinical isolates. Here we confirm such a result for clinical isolates obtained from patients treated in Argentinean hospitals. Moreover, we demonstrate that the expression of TTSS-associated proteins is independent both of the year in which the isolate was obtained and of the types of polymorphic alleles for other virulence factors but is dependent on environmental growth conditions. Interestingly, we observed that TTSS-associated protein expression is lost after successive *in vitro* passages but becomes operative again when bacteria come into contact with the host. This *in vivo* activation of TTSS expression was observed not only for clinical isolates previously adapted to the laboratory after successive *in vitro* passages but also for vaccine strains that did not express the system *in vitro*. The reversibility of TTSS expression, demonstrated by its switching off-on when the bacterium comes into contact with the host, appears to be an adaptive response of this pathogen.

INFECTION AND IMMUNITY, Mar. 2008, p. 1257–1266

Bordetella pertussis Expresses a Functional Type III Secretion System That Subverts Protective Innate and Adaptive Immune Responses

Neil K. Fennelly,.....and Kingston H. G. Mills

secretion of the *Bordetella* TTSS tip fibrillar protein Bsp22 by a significant portion of **low-passage clinical isolates** of *B. pertussis,*

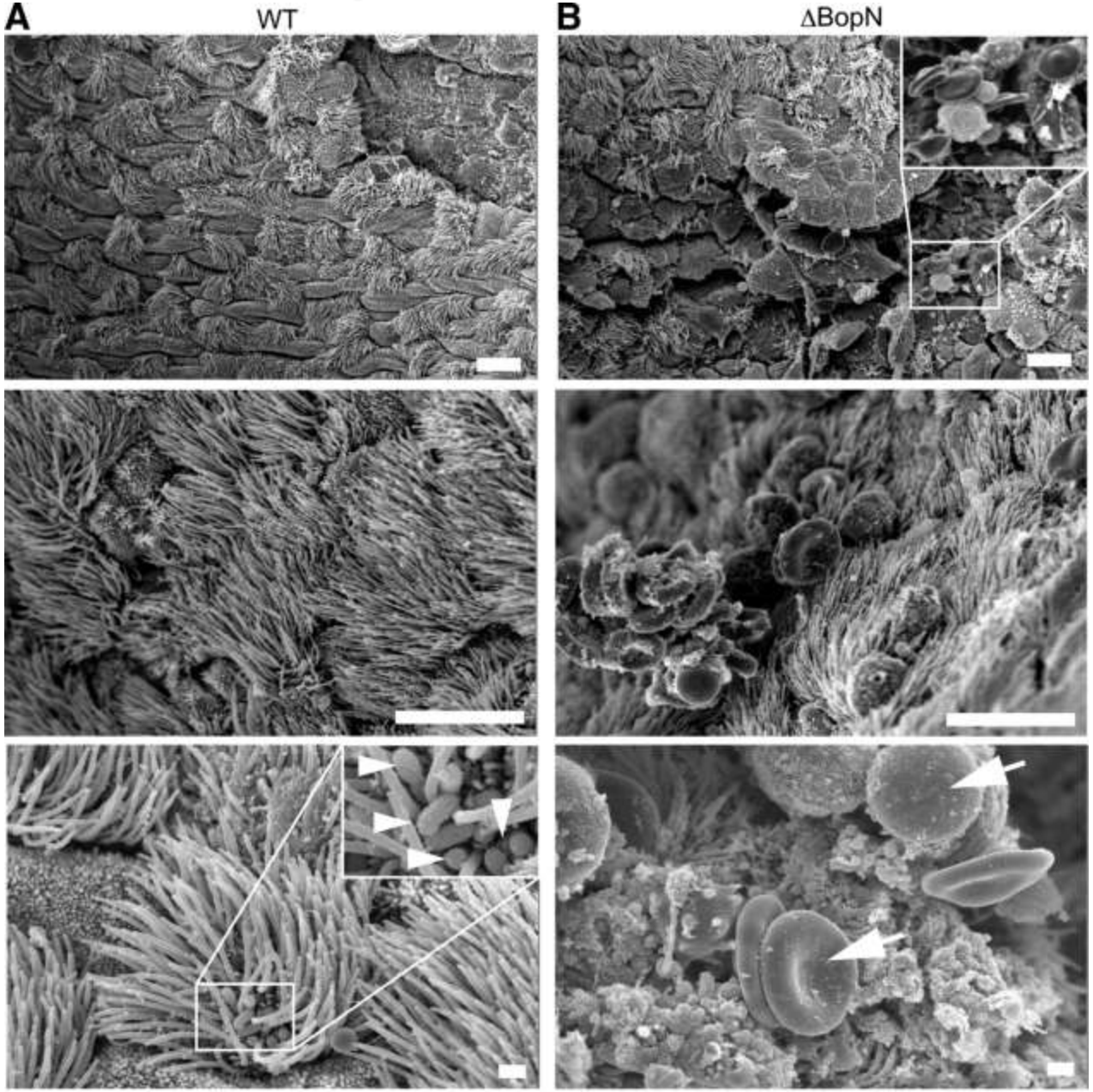
But not by common laboratory-adapted strains, such as Tohama I and Wellcome 28.

Mutation of *bscN* abolished *in vitro* secretion of TTSS substrates
by a clinical isolate of *B. pertussis,*

BopD and Bop N secreted?, BteA (BopC) not identified as secreted by the tested strains (fits with later reports)

***bscN* mutants exhibit reduced ability to colonize the respiratory tracts of mice, enhanced local inflammatory and antigen-specific cellular and humoral immune**

in *B. broinchiseptica* infections the BopN suppresses inflammatory responses at the bacteria-colonized epithelia



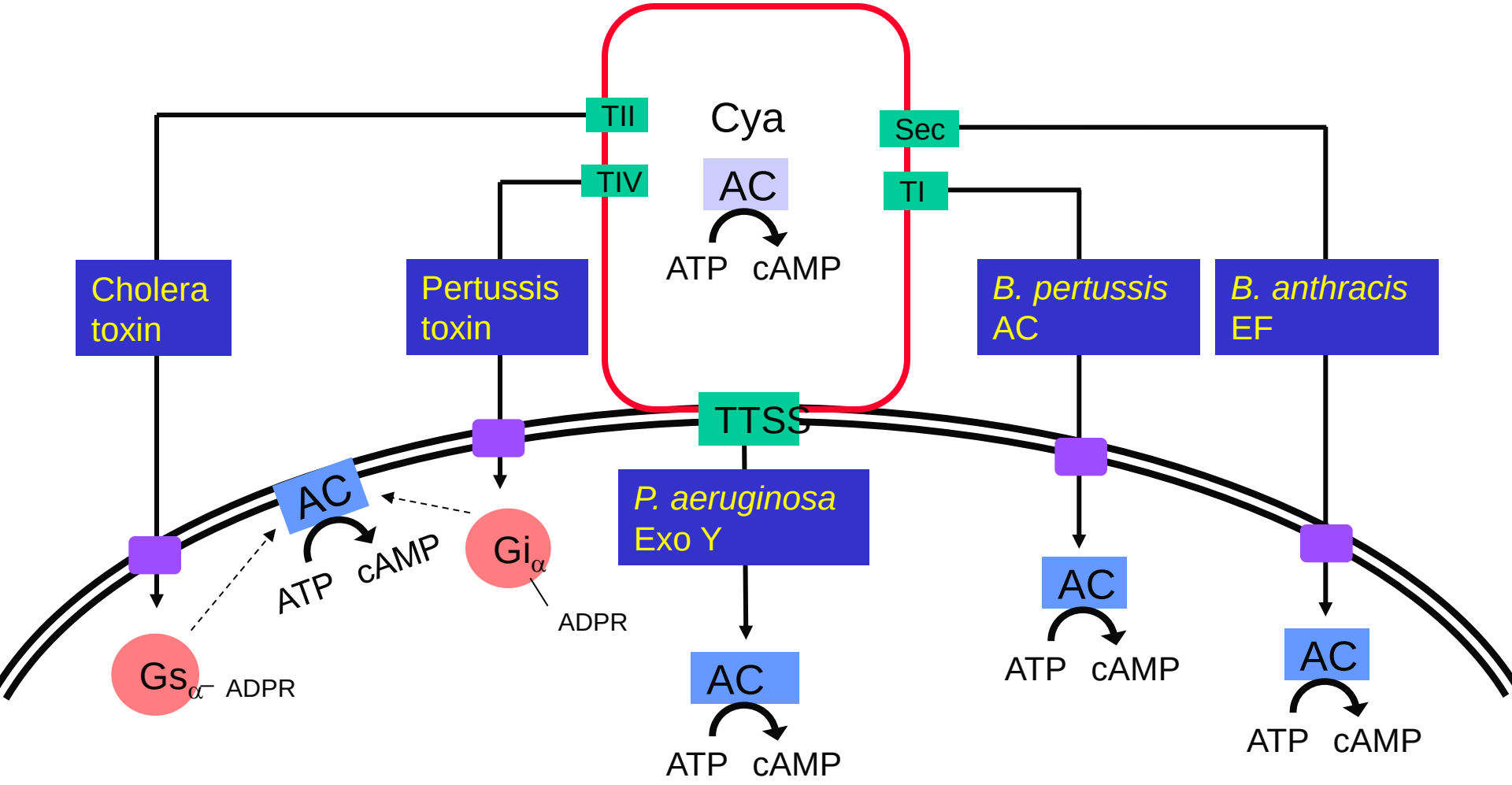
(A and B) Scanning electron micrographs of mouse tracheas infected with WT (A) and ΔBopN *Bordetella bronchiseptica (B).*

C57BL/6J mice were infected intranasally with 5×10^6 WT and ΔBopN B. *ronchiseptica,* and tracheal sections were obtained 2 d after infection.

Note that **extensive cell-surface disruption,** including **increased unciliated cells** as well as **infiltration of inflammatory cells** and erythrocytes, is observed in mice infected **with ΔBopN but not WT.**

The ‘smartest’ toxins subvert cell signaling

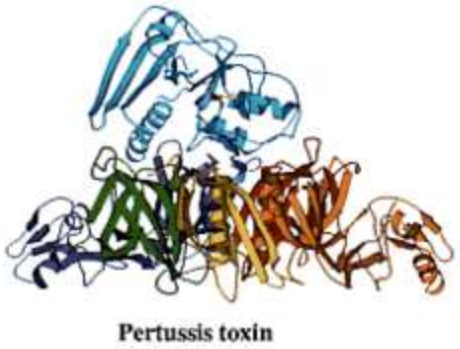
Such as fooling cells by cAMP – the second messenger…!

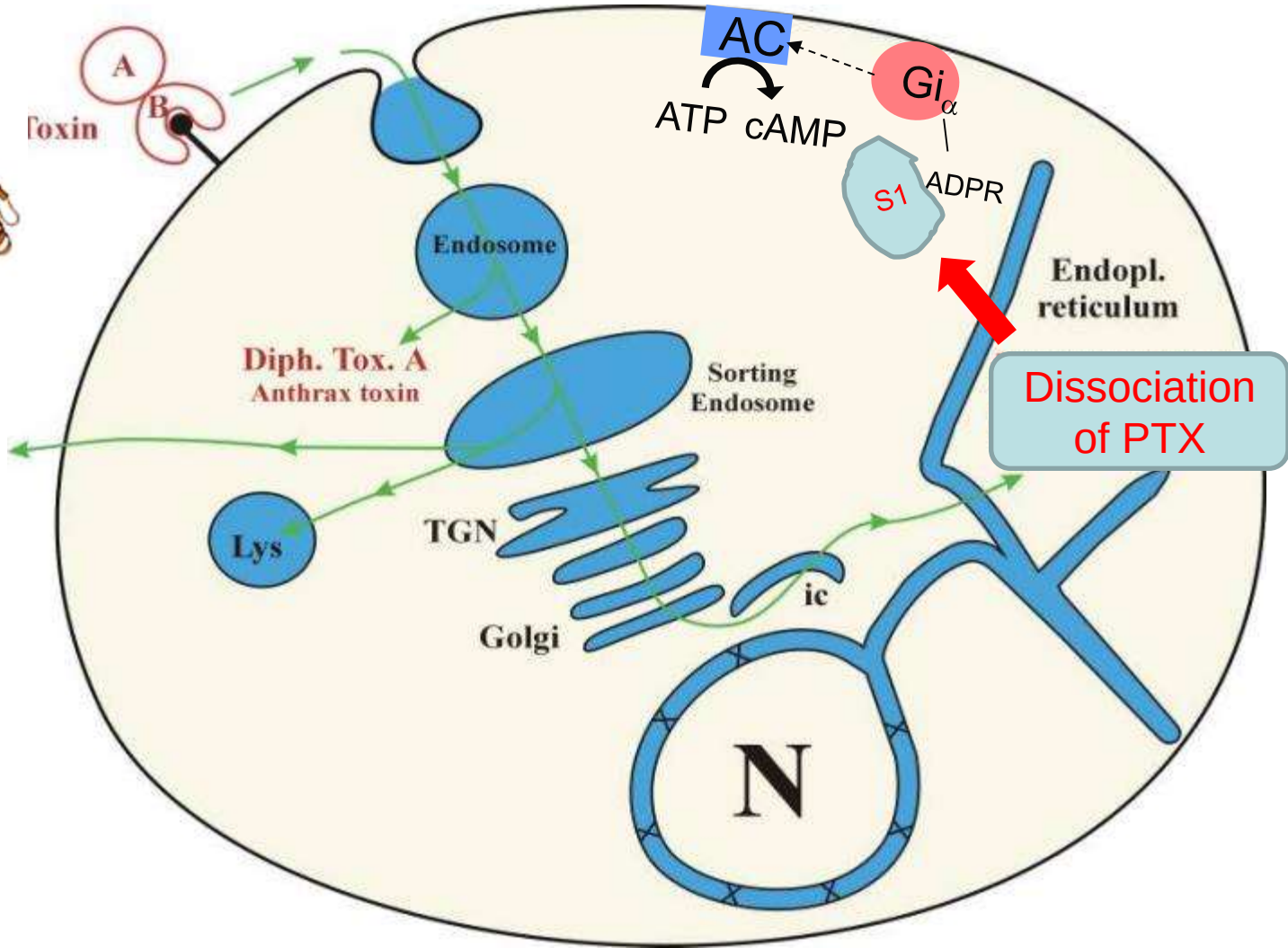


Slide: courtesy of S. Lory, Hravard Medical School

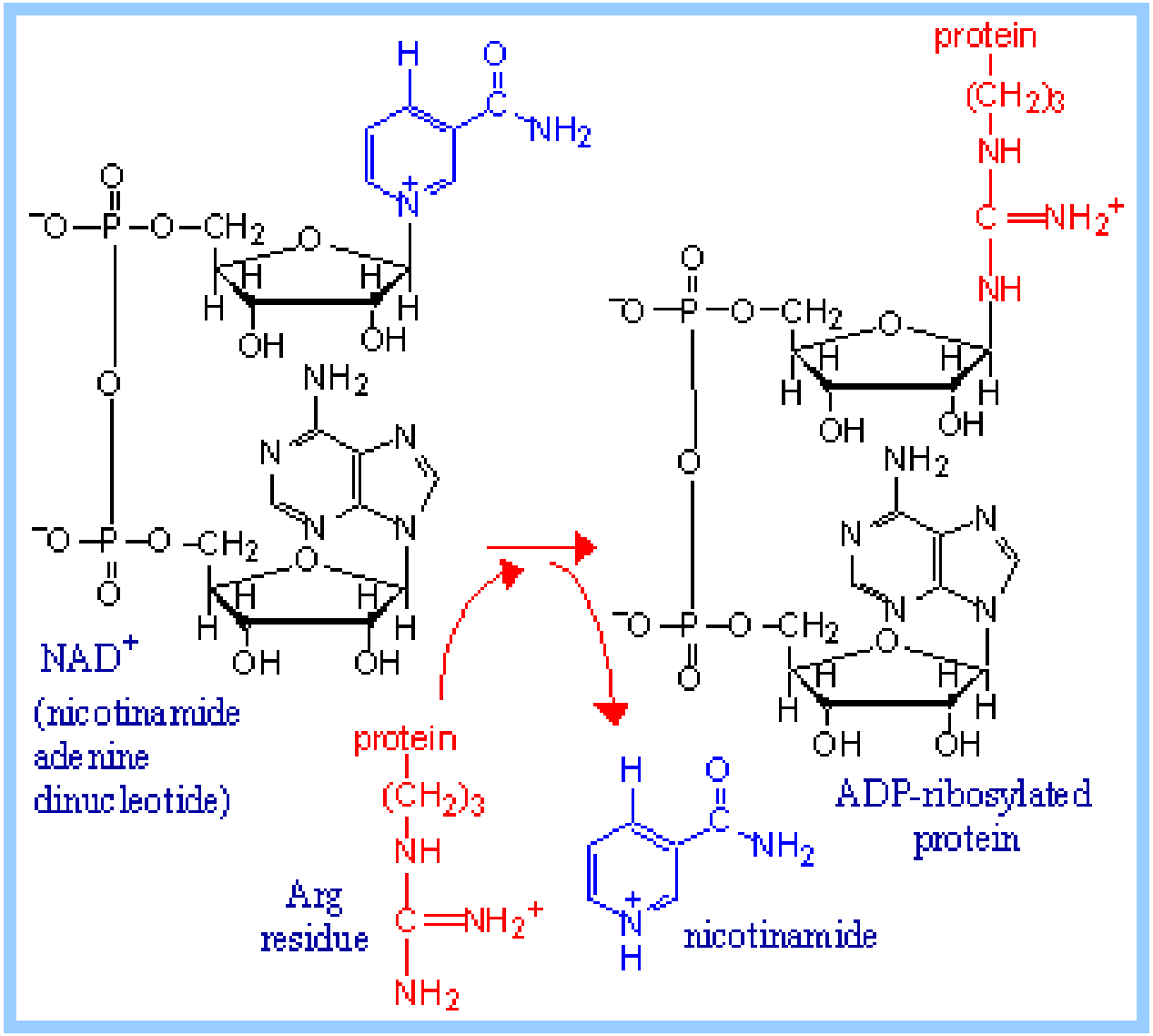
Pertussis toxin (PT),

an AB_5 exotoxin is trafficked along a retrograde transport pathway , through the Golgi complex to endoplasmic reticulum (ER), where dissociation of the holotoxin l occurs. S1 then translocates to cytosol and where it ADP-ribosylates its Gi protein targets.





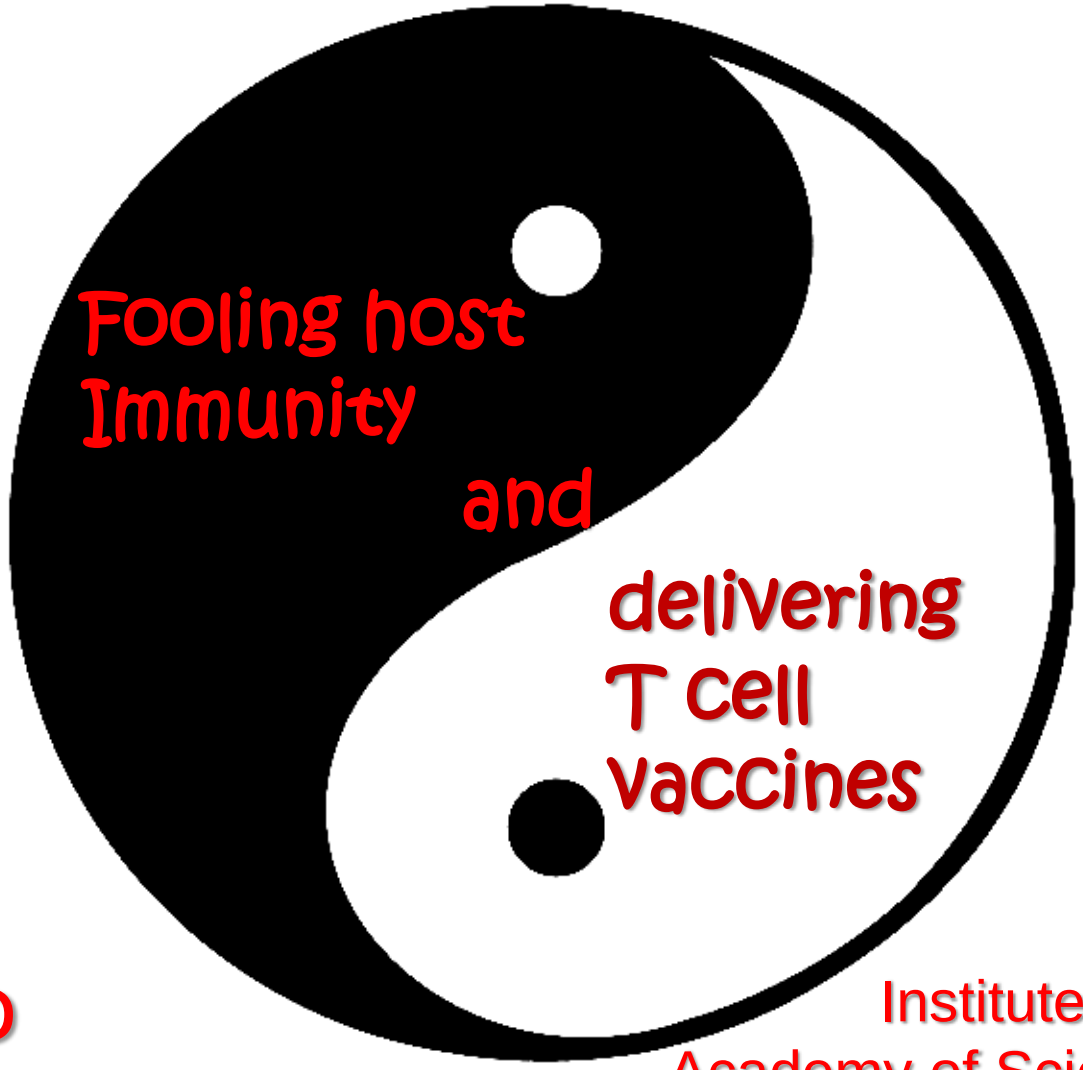
Pertussis toxin catalyzes ADP-ribosylation of Gi proteins



Pertussis questions to be asked in baboons

- Current aP vaccines were developed to protect MICE against an exclusively human pathogen…
  - Pertussis vaccine testing in baboons now becomes a must…
  - Significant interest of companies, including us, limited facility capacities and accessibility, high cost
- **Open questions to be answered:**
- many genes of unknown function = examine their role in virulence = can do functional genomics in a relevant animal model now… = STM or Tnseq, RNAseq (can get BAL fluids for example)
- How does the pathophysiology of whooping cough really look like in vivo????
- **Why do we cough?** What is the cough toxin?
- Is the long coughing period due to epithelial damage or cell invasion? How is it with mucus production in whooping cough???
- How does the histology change in time in different locations = where are the bacteria and what do they produce as virulence factors = what does it do to the tissue?
- Is there an intracellular phase of life of *B. pertussis* in human cells?
  - If yes, which cells? – phagocytes or epithelial cells? – some evidence exists *ex vivo* and *post mortem*
- What is the role of the T3SS in *B. pertussis* virulence? How is it regulated in vivo? Where does it express on the mucosa, inside phagocytes, or not at all?
- Does *B. pertussis* phase variation occur *in vivo*? Where and when? What for?

THE YIN AND YANG OF A BACTERIAL TOXIN



P. Šebo

PRAGUE
Institute of Microbiology
Academy of Sciences of the CR

Real title of this talk:

What we eventually may believe
to have learned on

Adenylate cyclase toxin

role in subversion of host immunity
and
in the pathogenesis of pertussis

in mice…

A bit of history - I.

1976 ACT discovered by Hewlett EL, Urban MA, Manclark CR, Wolff J.: 'Extracytoplasmic adenylate cyclase of *Bordetella pertussis*'. PNAS 73:1926-30.

1977 Hewlett EL, Manclark CR, Wolff J:. Adenyl cyclase in Bordetella pertussis vaccines. J Infect Dis. 1977 Aug;136 Suppl:S216-9

1980 Wolff *et al.:* Calmodulin activates prokaryotic adenylate cyclase. PNAS 77: 3841

1982 Confer DL and Eaton JW: Phagocyte impotence caused by **an invasive** bacterial adenylate cyclase. Science 217:948:

...For unknown reasons, humans infected with the bacterium Bordetella pertussis are exceptionally vulnerable to secondary infections. Bordetella species elaborate a soluble, heat-stable, and highly active adenylate cyclase. This enzyme is internalized by phagocytic cells and catalyzes the unregulated formation of adenosine 3',5'-monophosphate (cyclic AMP), thereby disrupting normal cellular function. This unusual phenomenon ***may explain Bordetella-induced aphylaxis..***

aphylaxis = absence of phylaxis or immunity

Obsolete term meaning lack of protection against disease

Lack of protection against disease. Also called *nonimmunity*.

Confer DL and Eaton JW, Science 217:948

Phagocyte impotence caused by **an invasive** bacterial adenylate cyclase. :

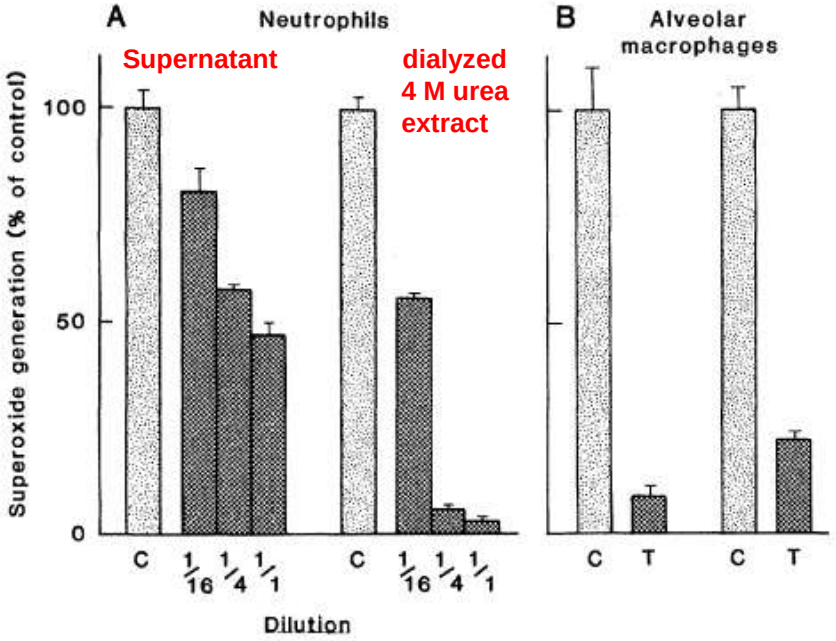


Fig. 1. Superoxide generation by stimulated human phagocytes and inhibition by *Bordetella* products. (A) Human neutrophils, 2×10^6, suspended in 200 μl of Hanks balanced salt solution, were incubated for 10 minutes at 37°C with 200 μl of the indicated dilution of the supernatant of 48-hour cultures of *B. pertussis* (protein content, 120 μg/ml) (left panel) and with dialyzed extract of *B. pertussis* organisms (protein content, 520 μg/ml) (right panel). Cytochrome *c* (1.2 mg) and opsonized zymosan (1 mg) were added (total volume, 1 ml) and the superoxide-dependent reduction of ferricytochrome *c* was determined after 10 additional minutes of incubation at 37°C as previously described (*8*). Results are expressed as percentages of control (untreated values), and bars represent the range of independent triplicate determinations. (B) Human alveolar macrophages (10^6) suspended in 100 μl of Hanks balanced salt solution were incubated with 100 μl of dialyzed extract of *B. pertussis* (*T*) or 100 μl of external dialysis fluid (*C*) as above. The cells were then stimulated by the addition of 1 mg of opsonized zymosan (left bars) or 0.1 μg of phorbol myristate acetate (right bars). Superoxide production was assessed by following luminol-enhanced chemiluminescence as described (*11*). Results represent the mean and range of triplicate determinations.

Culture supernatants contain very little of active ACT = huge potency!

Sample	Treatment	Cyclic AMP (pmole/ 10^7 PMN)	Adenylate cyclase (pmole/10^7 PMN-min)
Neutrophils	Incubated for 20 minutes at 37°C, washed, trypsinized, washed, homogenized	4.9	0, 0*
Neutrophils plus *B. pertussis* extract (540 μg/10^7 PMN)	Incubated for 20 minutes at 37°C, washed, trypsinized, washed, homogenized	1296	41.9, 28.0, 45.1
Neutrophils plus *B. pertussis* extract (540 μg/10^7 PMN)	Incubated for 20 minutes at 0°C, washed, trypsinized, washed, homogenized	6.7	4.2, 4.3, 4.8

*Limit of detection, < 1 pmole per 10^7 PMN per minute.

Confer DL and Eaton JW, Science 217:948

Phagocyte impotence caused by an invasive bacterial adenylate cyclase. :

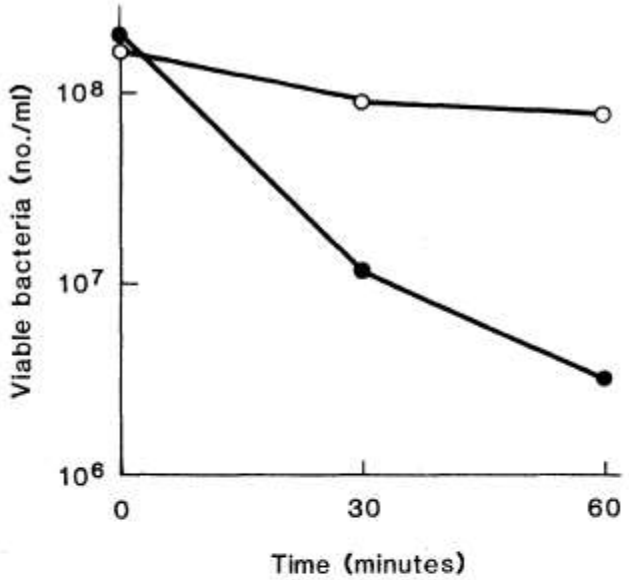


Fig. 2. Neutrophil killing defect induced by *Bordetella* extract. Human neutrophils (2×10^7 per milliliter) suspended in Hanks balanced salt solution were incubated for 5 minutes at 37°C with an equal volume of dialyzed *Bordetella* extract or dialysate control. The killing of *Staphylococcus aureus* 502A was assessed as described (*12*) by admixing 5×10^6 neutrophils, 2×10^8 bacteria, and 0.1 ml of pooled human serum in a total volume of 1 ml. Numbers of viable bacteria remaining were determined by plating dilutions of the incubation suspension removed at 0, 30, and 60 minutes. Each point represents the mean of quadruplicate determinations. Control tubes containing no neutrophils showed no change in bacterial count. Symbols: ○, *Bordetella*-treated neutrophils; ●, control neutrophils.

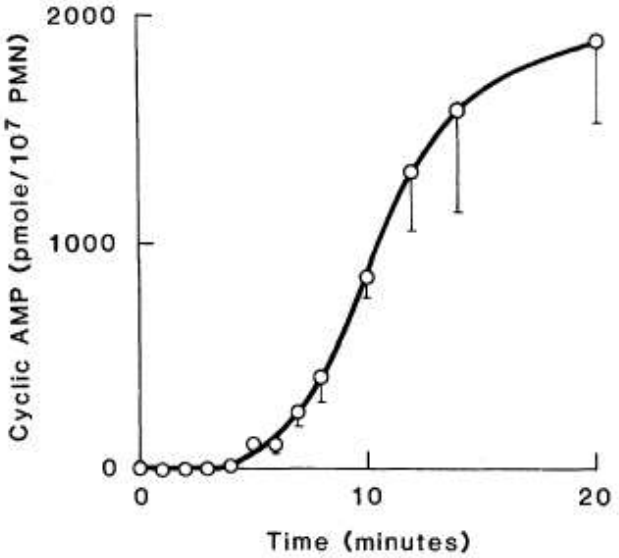
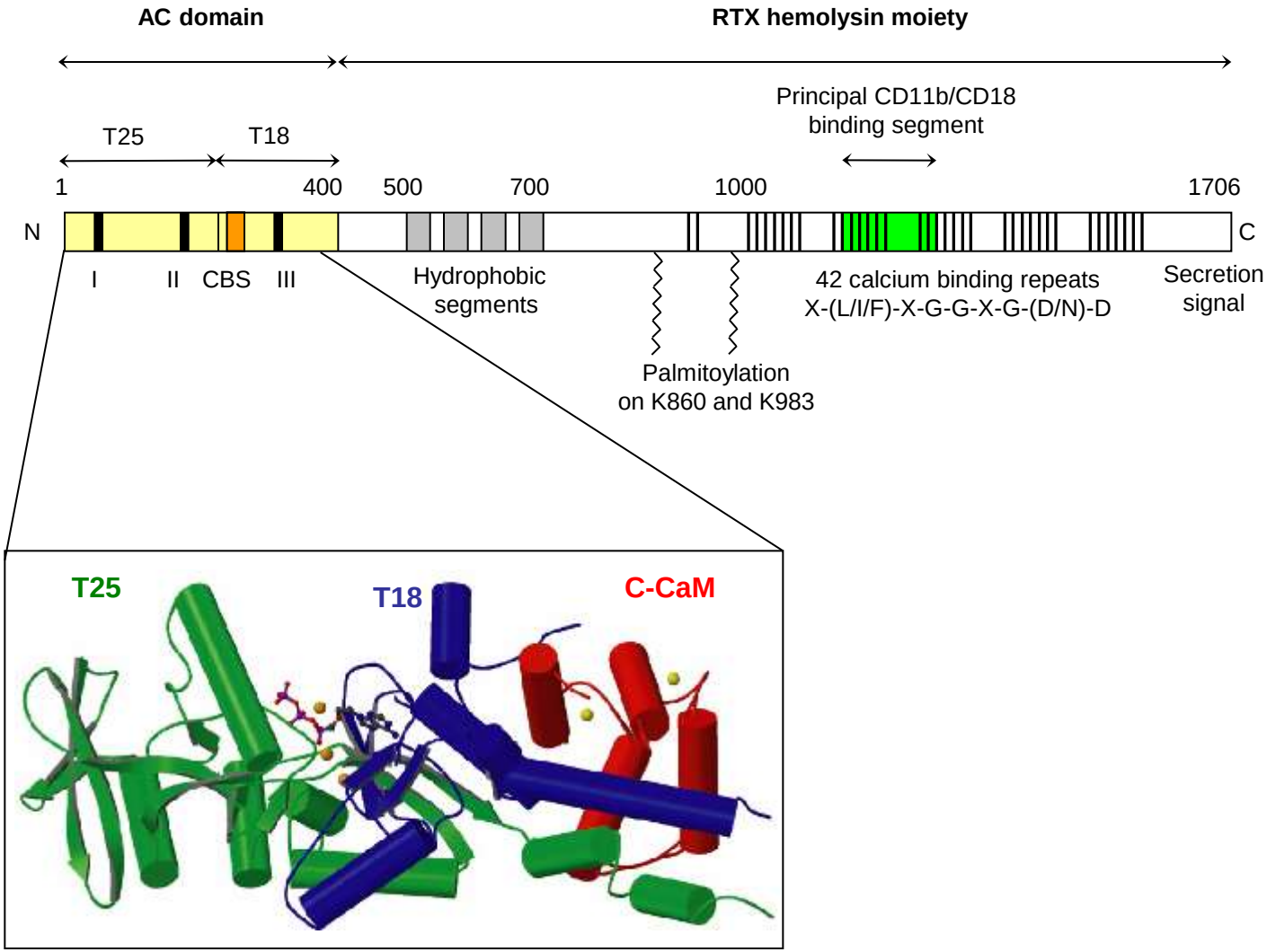


Fig. 3. Accumulation of cyclic AMP in human neutrophils (*PMN*) incubated with dialyzed *Bordetella* extract. Neutrophils, 10^7 per milliliter in Hanks balanced salt solution, were incubated at 37°C with equal volumes of dialyzed *Bordetella* extract (protein content, 520 μg/ml) for the times shown. Total cyclic AMP was determined as described (*16*). Values shown represent the means and standard error of seven separate (duplicate) determinations with neutrophils from four different donors. Normal neutrophils contain 2 to 5 pmole of cyclic AMP per 10^7 cells, and these amounts do not change during control incubations. Separate experiments (not shown), in which neutrophil pellets were obtained by brief centrifugation after incubation, indicated that > 90 percent of the total recoverable cyclic AMP is associated with the cell pellet.

Adenylate cyclase toxin - cytolysin

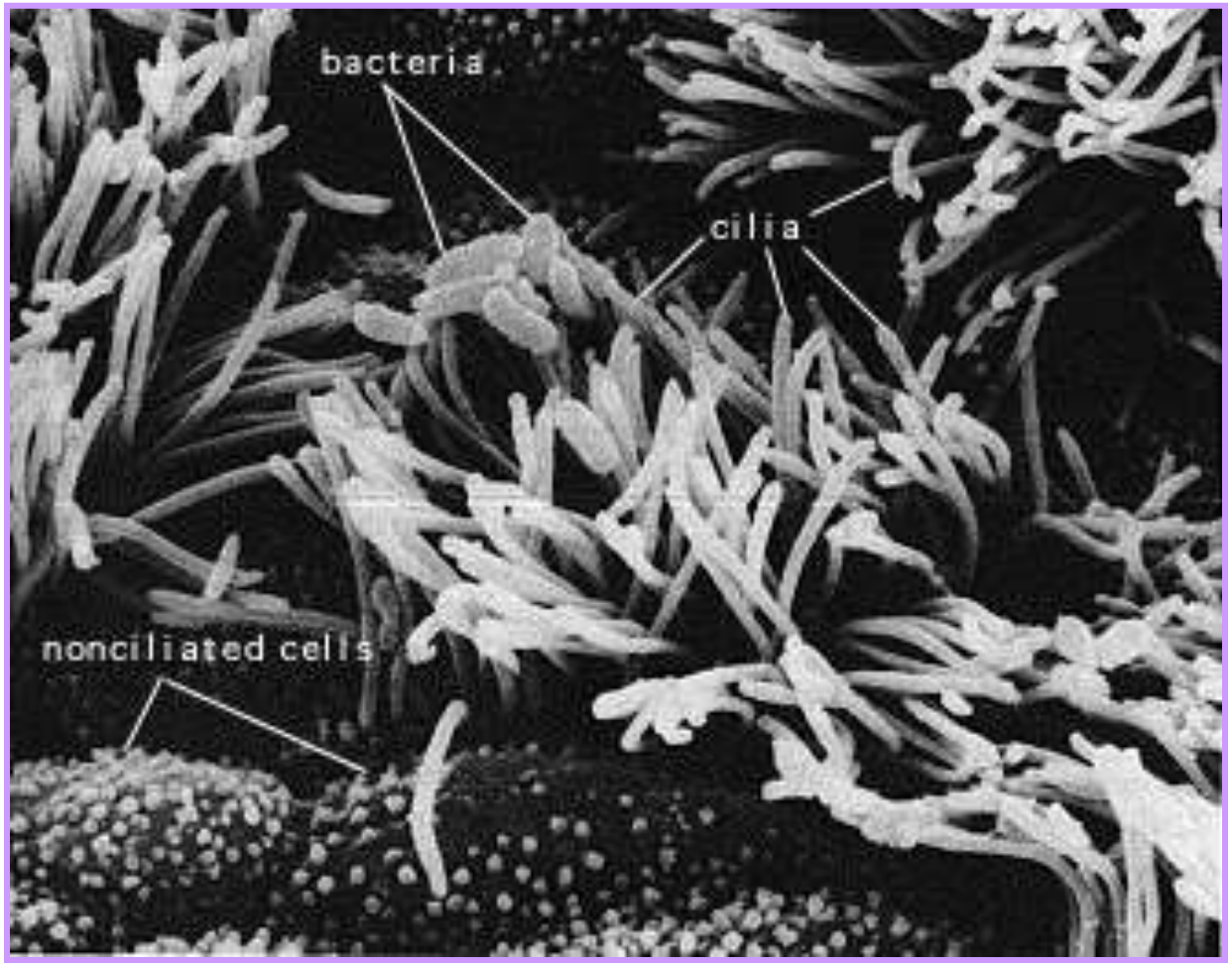


Guo Q. *et al.* (20005) EMBO J. 24, 3190–3201

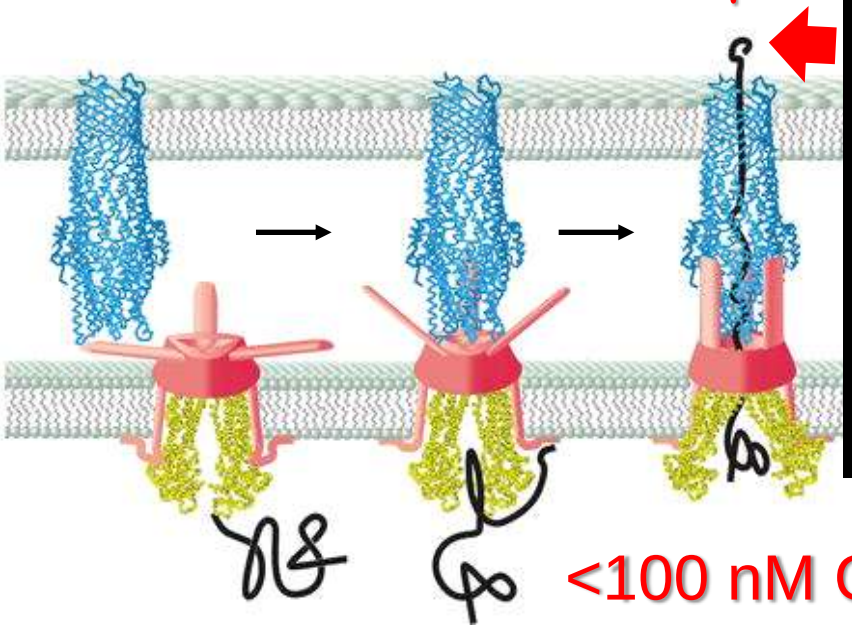
Adenylate cyclase toxin is critical for colonization *by Bordetella pertussis*

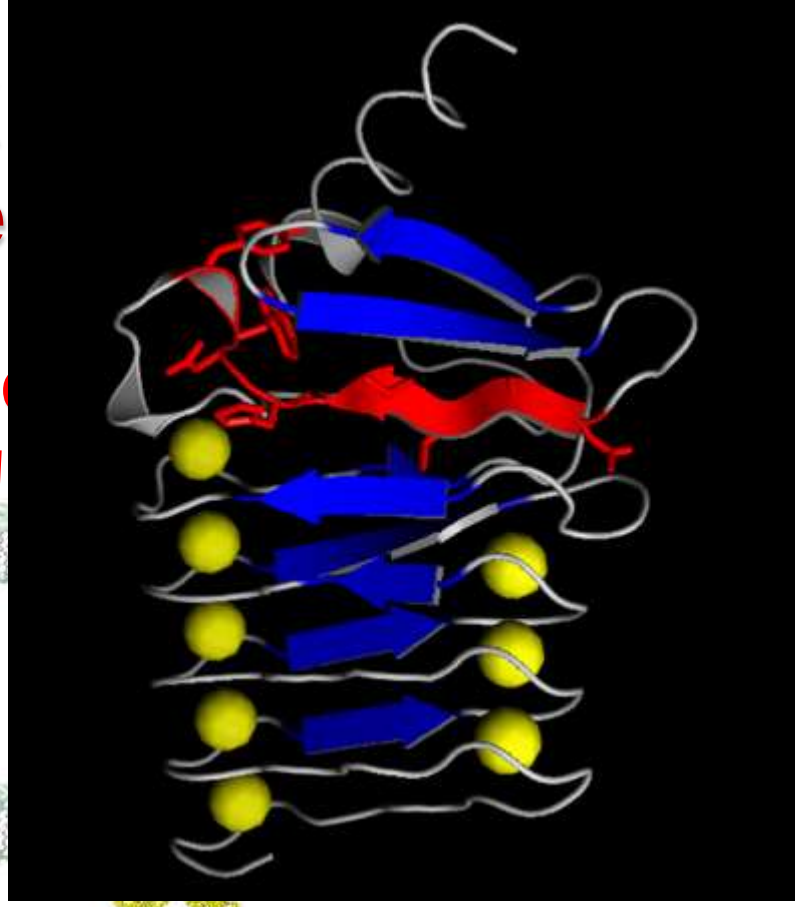
and pertussis toxin is critical for lethal infection in infant mice

Goodwin MS, Weiss AA. (1990) *Infect Immun*. 58:3445-7
Khelef N, Sakamoto H, Guiso N. (1992) Microb. Pathog. 12:227-35



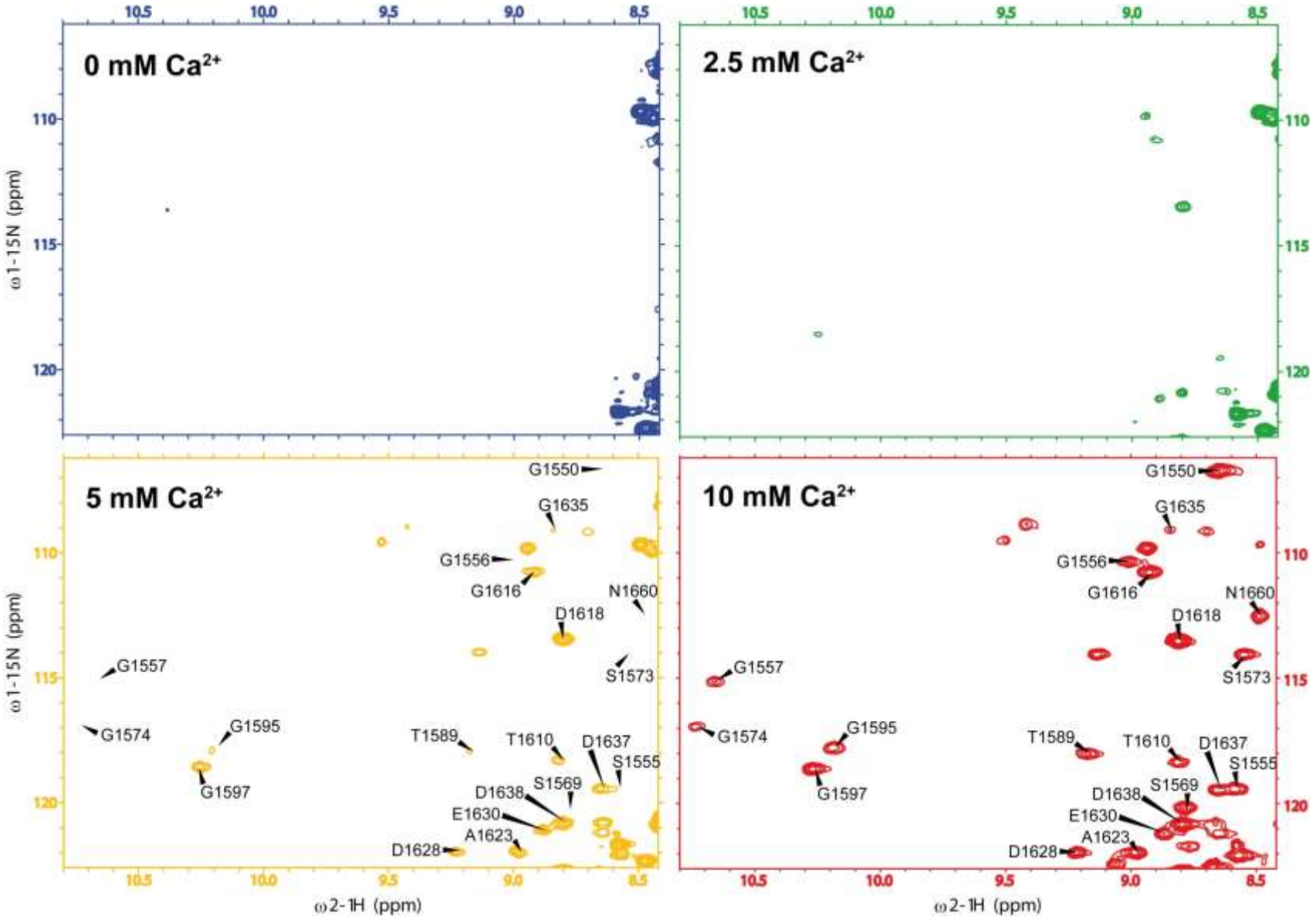
ACT is an RTX
secreted by a type



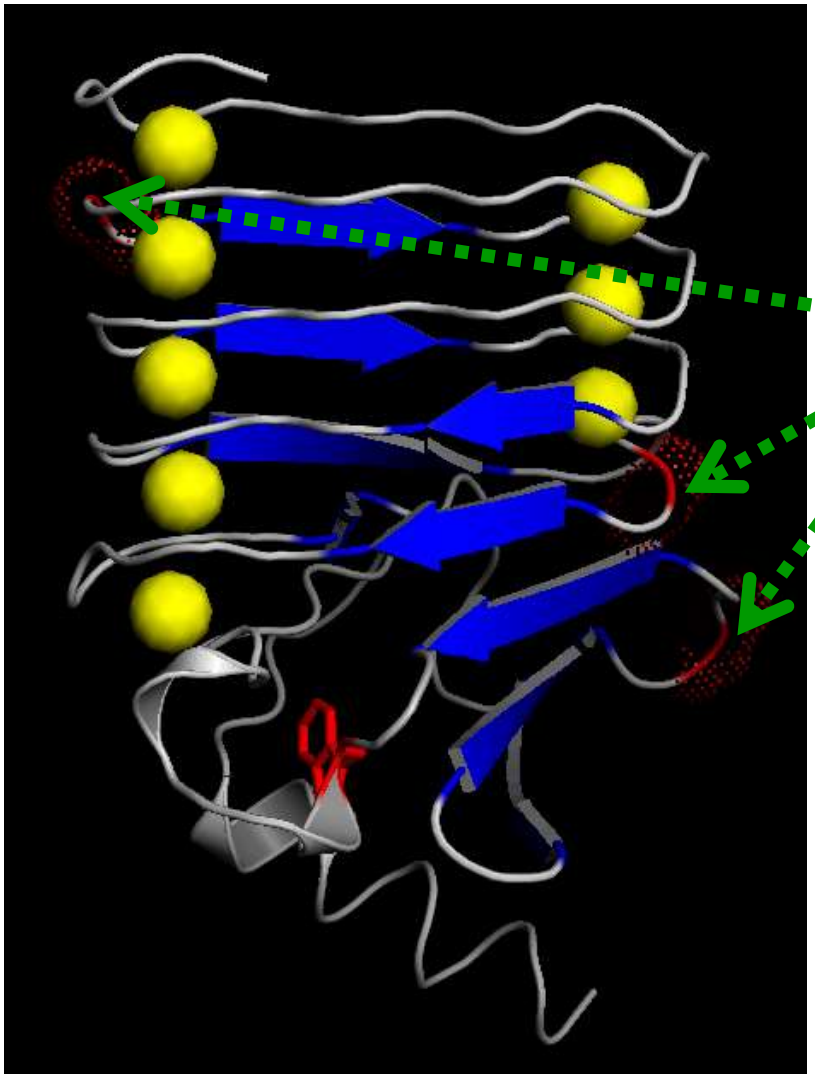


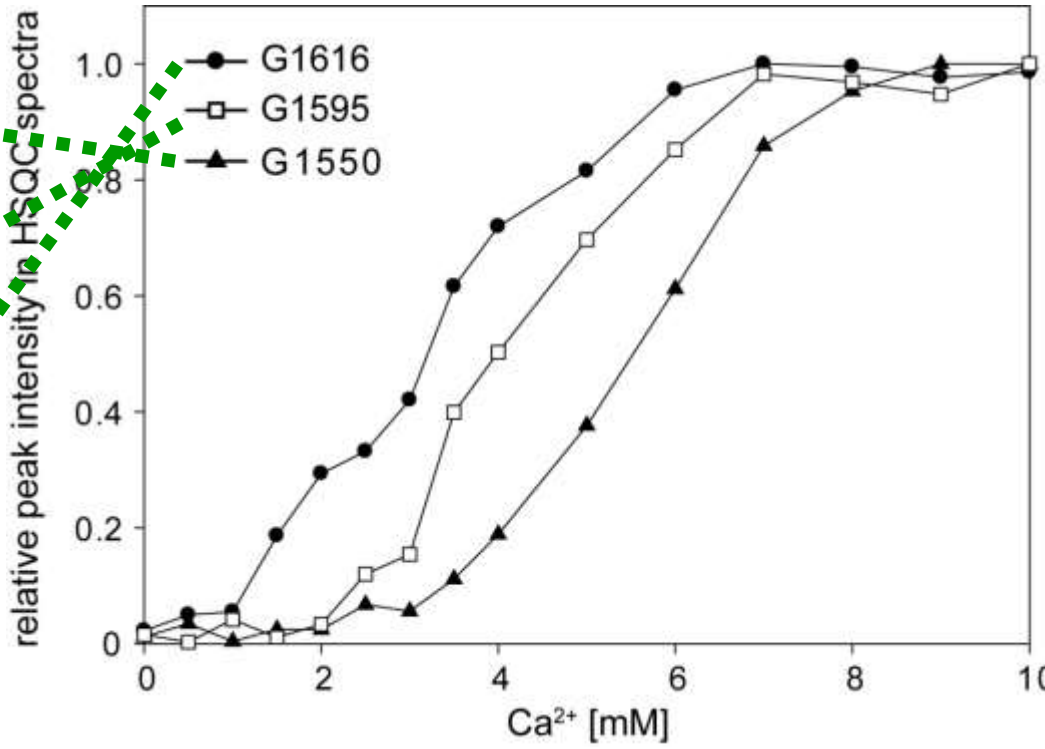
Need to unfold and refold
on the way to target…

Sequential folding of CyaA starts from the C-terminus of the protein (1530-1680) followed by by NMR (Ca^{2+} titration)

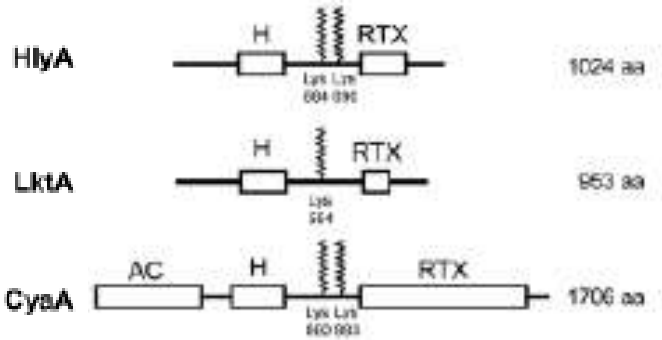


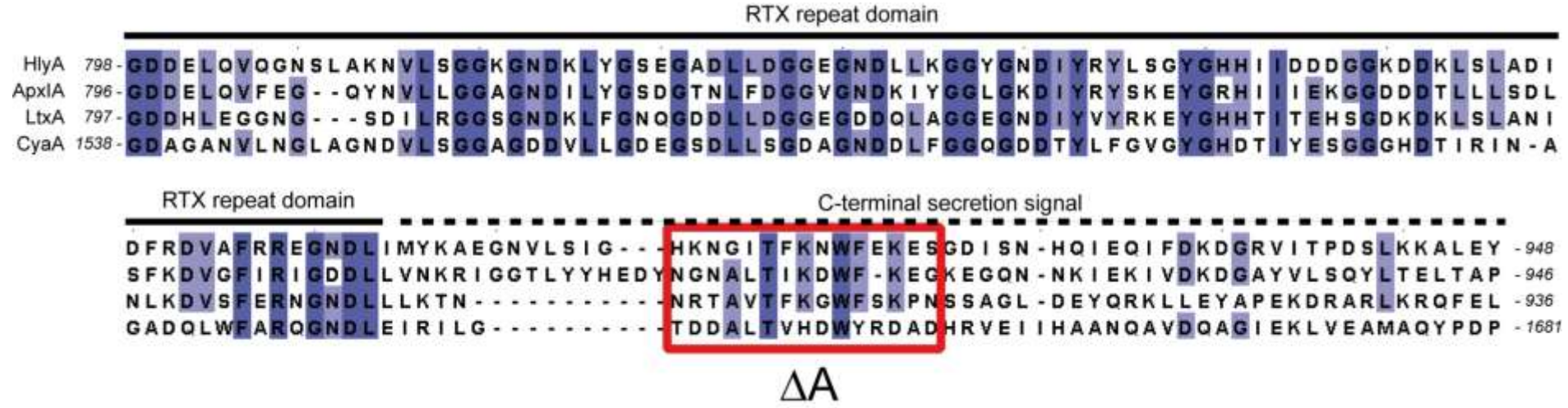
Sequential folding of CyaA starts from the C-terminus of the protein





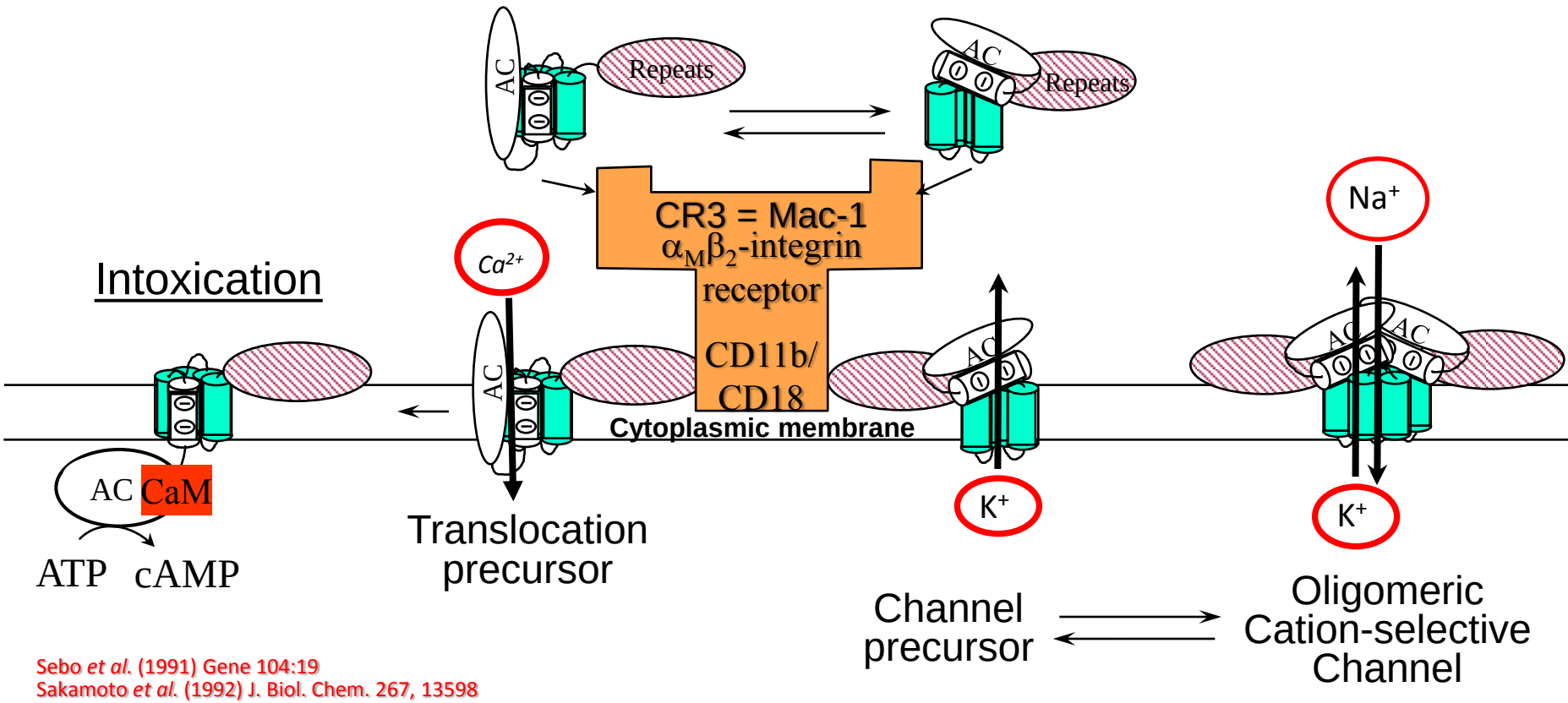
A conserved block of C-proximal residues is required also for activities of other RTX toxins





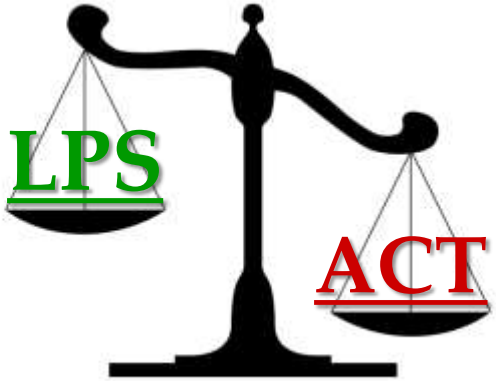
$_{*}T_{*}$+XWF - a conserved motif

The three cytotoxic activities of ACT adenylate cyclase toxin & pore-forming hemolysin/Cytolysin



Sebo *et al.* (1991) Gene 104:19
Sakamoto *et al.* (1992) J. Biol. Chem. 267, 13598
Benz *et al.* (1994) J. Biol. Chem. 269, 27231
Hackett *et al.* (1995) J. Biol. Chem. 270, 20250
Gray *et al.* (1998) J. Biol. Chem. 273, 18260
Osickova *et al.* (1999) J. Biol. Chem. 274, 37644
Basler *et al.* (2007) J. Biol. Chem. 282, 12419
Fiser R. *et al.* (2007) *J. Biol. Chem. 282, 2808*
Osickova *et al.* (2010) *Mol. Microbiol.* 75:15450-1562

ACT/cAMP signaling breakes the hell loose… and supresses TLR signaling of the bug…



↓ ciliary beating

defensins and other antimicrobial peptides:
hβdefensin2 ,
↓ βdefensin1,
↓ cathelicidin

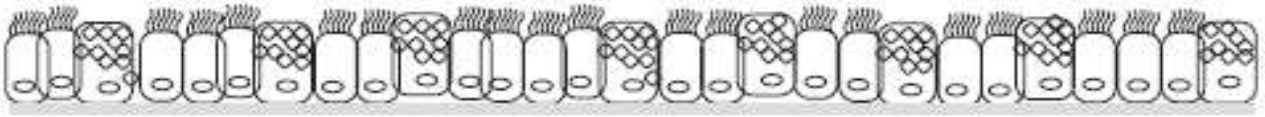
signal transduction events:
NF-κB, ↓ MAPK – p38, ERK, JNK

expression and upregulation of TLR:
TLR1-6, 9, TLR4, TLR2

mucin: MUC2, MUC5AC ↑

other soluble factors:
↓ O_2^-, NO, ↑ PGE2

AEC



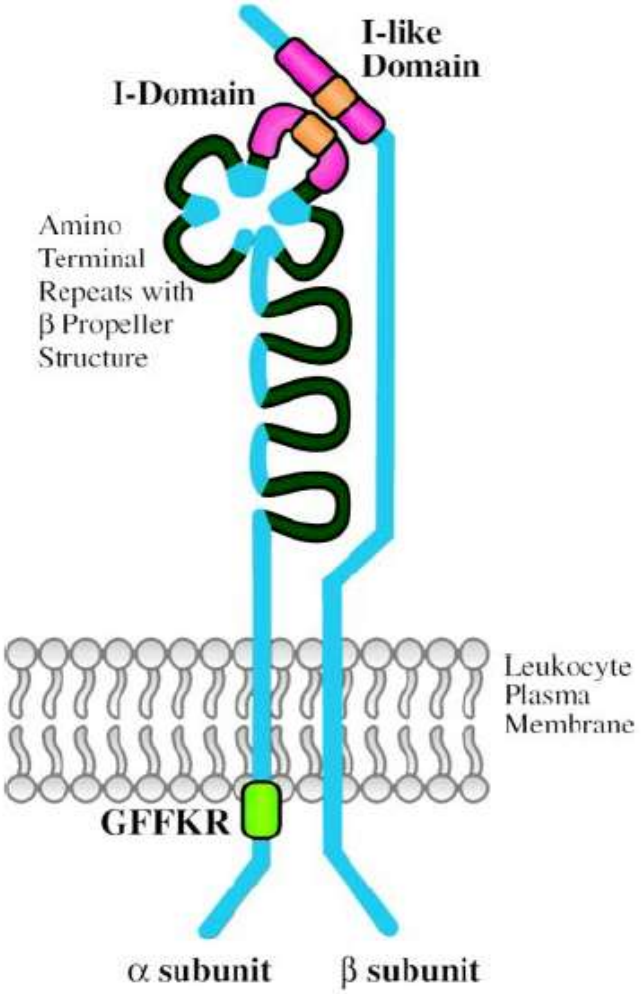
cAMP

other cells

cytokine and chemokines:
IL-1α, ↑ IL-1β, ↑ IL-6, ↑ IL-8, ↑ IL-10, ↓ TNFα, ↓ IFNβ, TGF-β, ↓ GM-CSF, MCP-1, ↓ MIP-1α, RANTES,..

expression of costimulatory x inhibitory molecules: ↑ CD80, CD86, ↓ CD40, ↓ CD54, B7-H2, B7-H3 x ↑ FasL, PD-L1, PD-L2

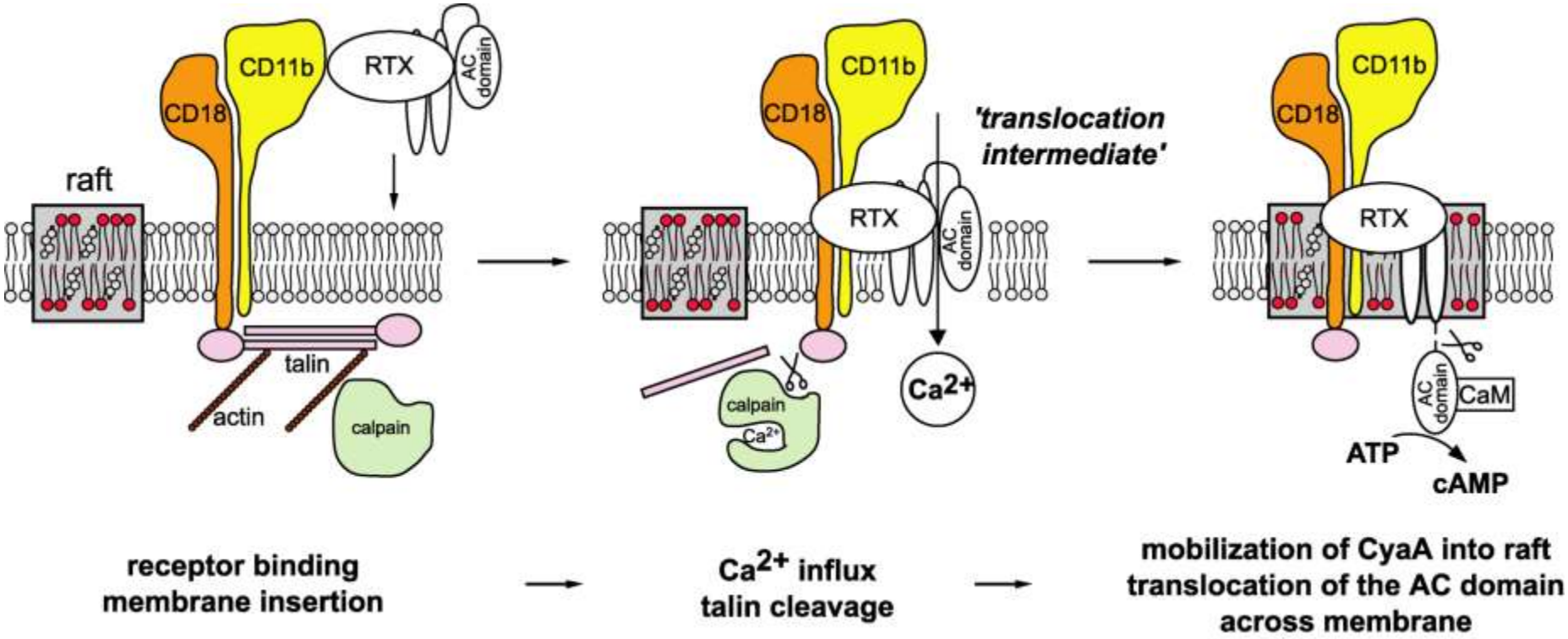
ACT targets myeloid phagocytes bearing $\alpha_M\beta_2$ integrin CD11b/CD18



- 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.

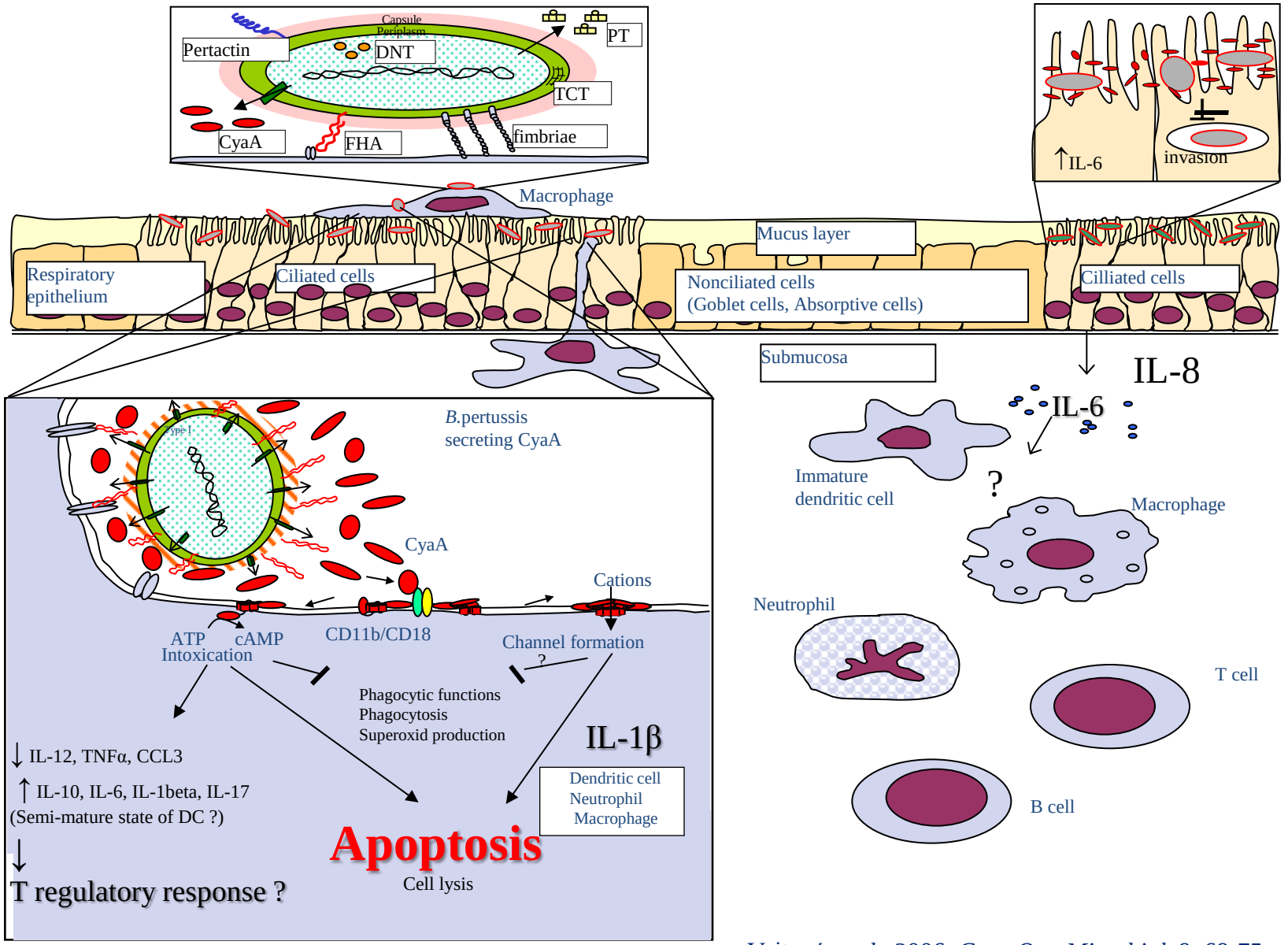
*A*denylate cyclase toxin hijacks the β_2 integrin receptor into lipid rafts to accomplish membrane translocation in two steps



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

ACT is a SWIFT SABOTEUR of immune responses

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



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

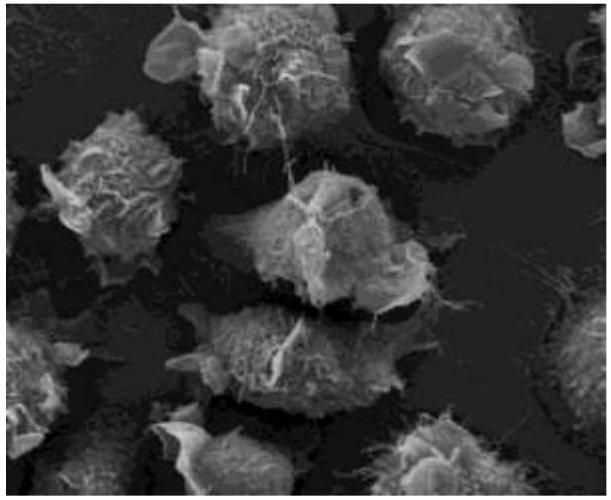
Vojtová *et al.*, 2006, *Curr. Op.. Microbiol.* 9, 69-75

CyaA-induced morphological rearrangements

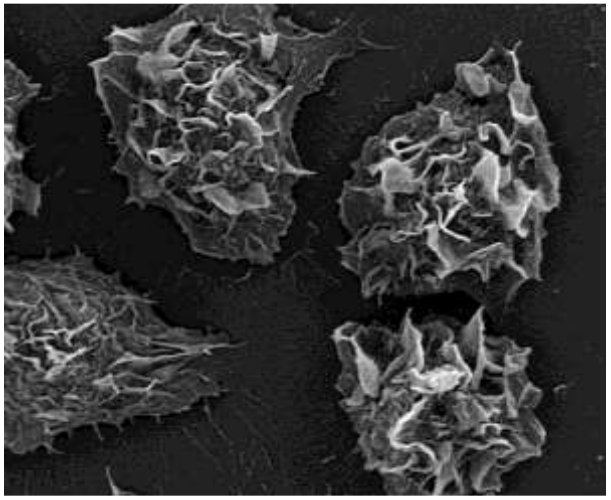
Mouse macrophage-like cell line J774 A.1:

Kamanova et al. (2008)
J. Immunol. 181, 5587-97

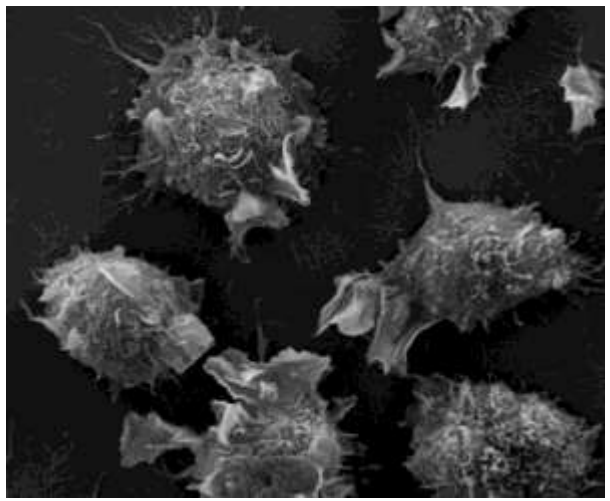
Buffer, 5 min



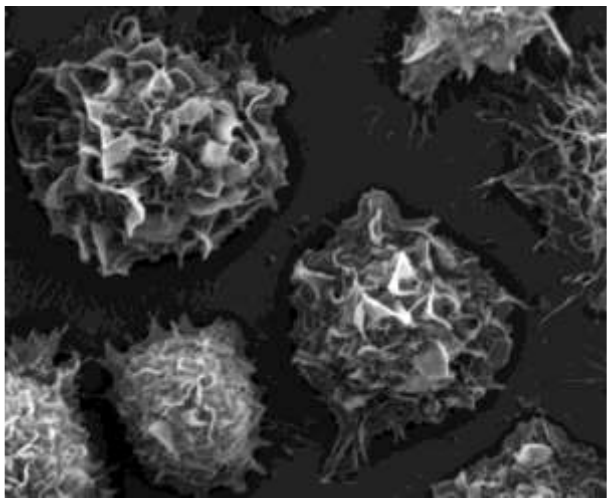
CyaA, **10 ng/ml,** 5 min



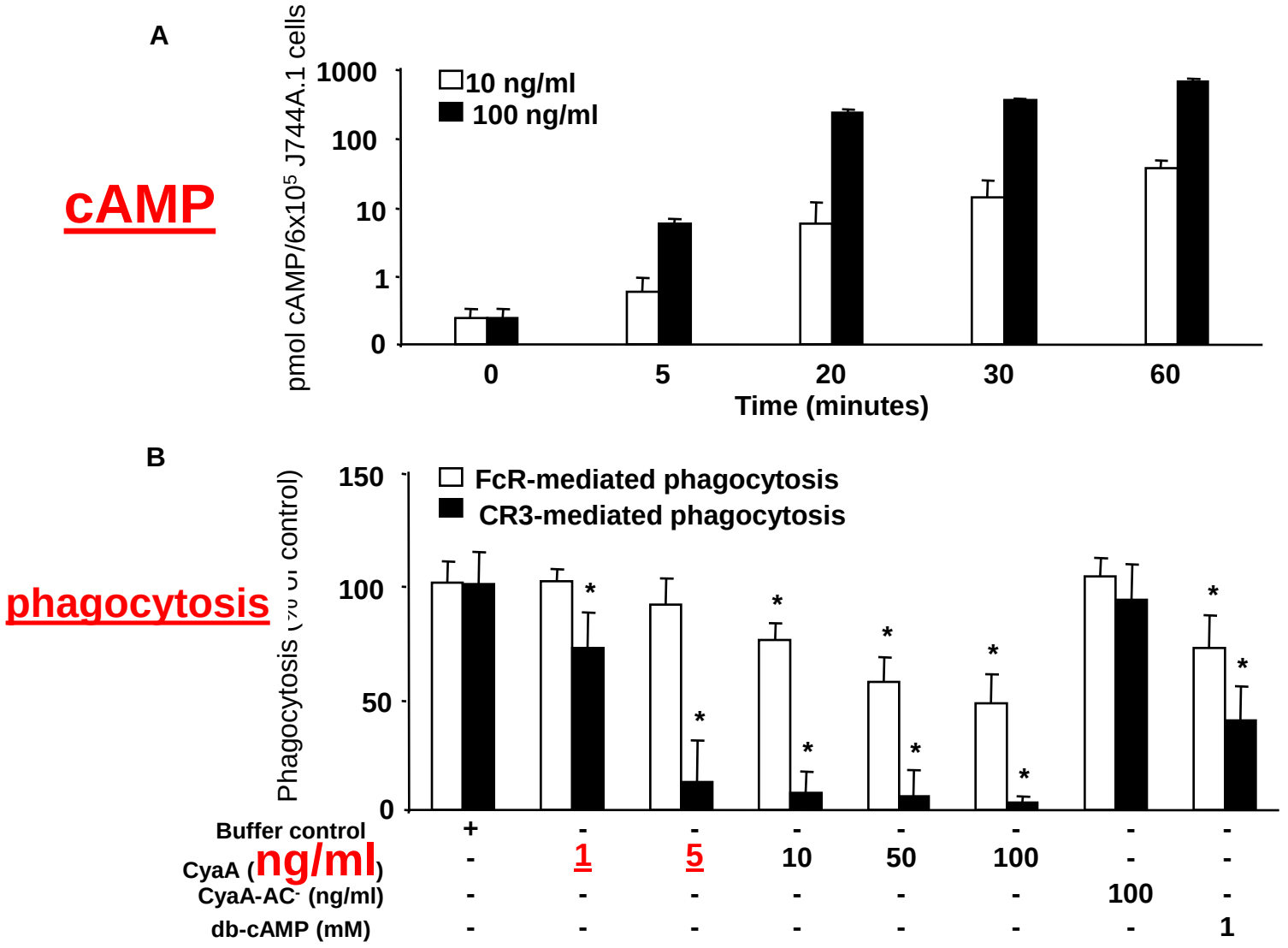
CyaA-AC^{-}, 10 ng/ml, 5 min



db-cAMP, 2mM, 10 min

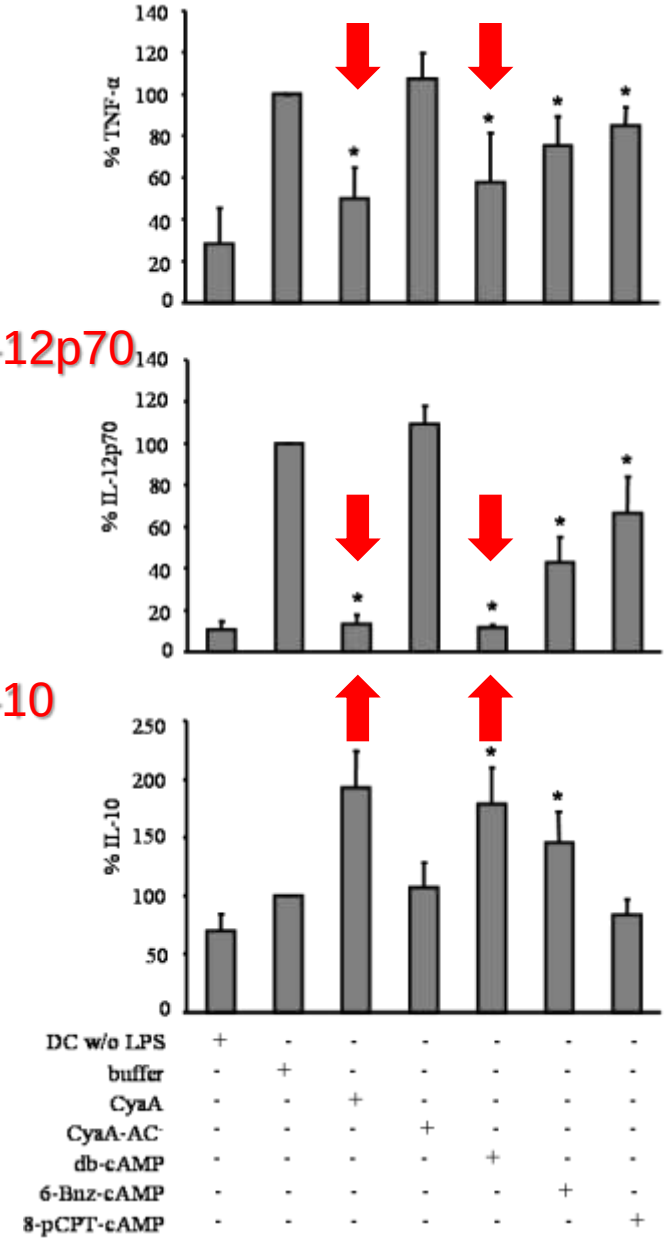


ACT at low doses ablates complement-mediated opsonophagocytosis (through RhoA inactivation)



Kamanova et al. (2008) *J. Immunol.* 181, 5587-97

TNFα



IL-12p70

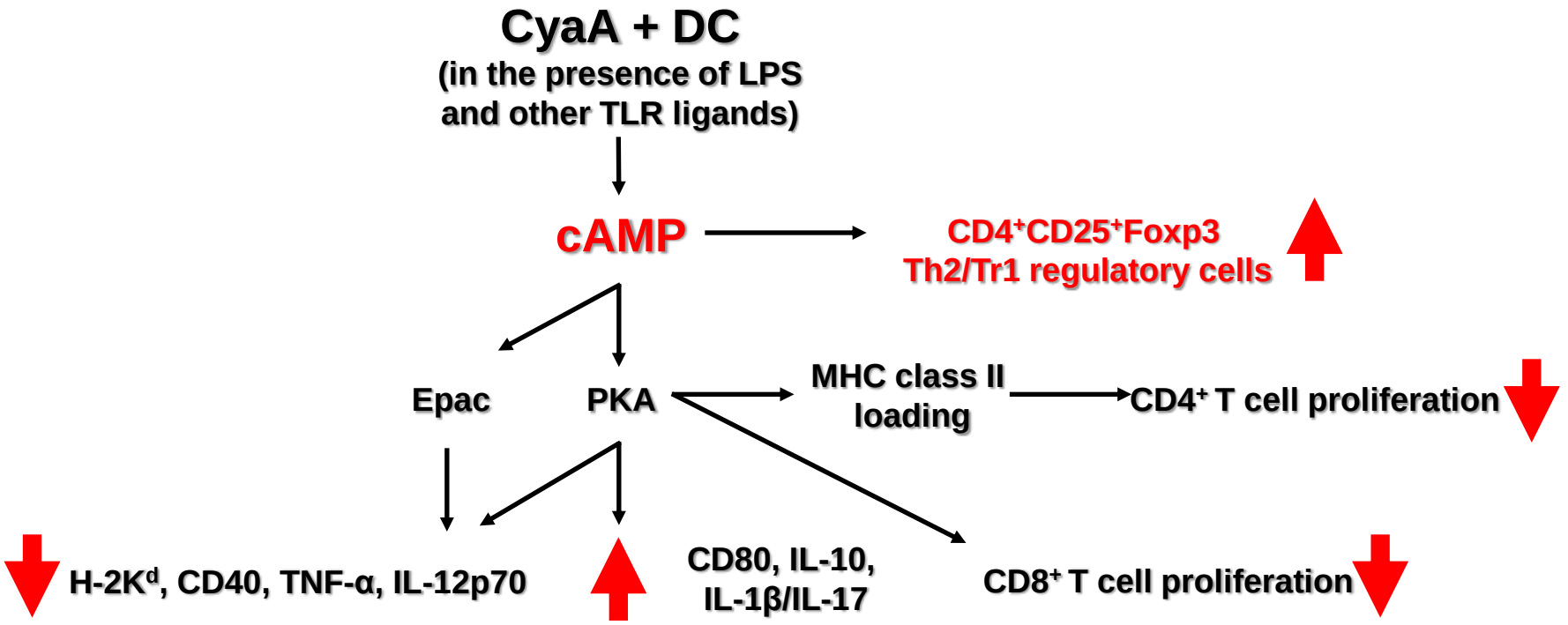
IL-10

ACT (CyaA) skewes TLR-stimulated cytokine production in DC towards tolerance?

n = 4, * P< 0,05; 100 % urea + LPS (buffer)

Irena Adkins

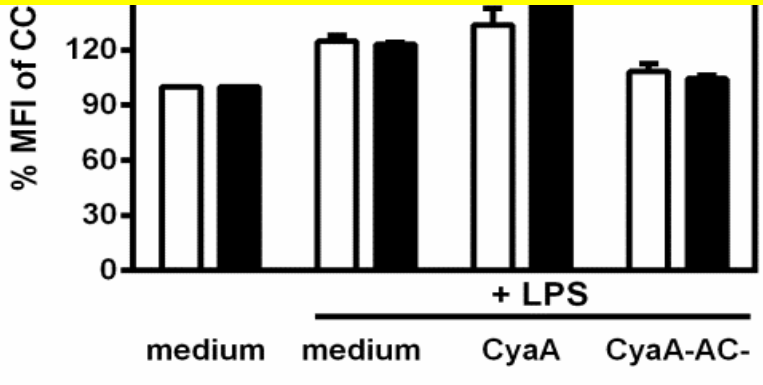
cAMP signaling of ACT dampens and skews adaptive immunity towards initial T_{h2}/T_{r1}-mediated tolerance of colonizing *Bordetellae* followed by delayed T_{h1}/T_{h17} mediated clearance?

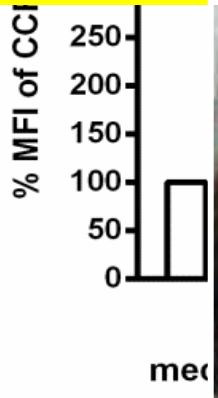


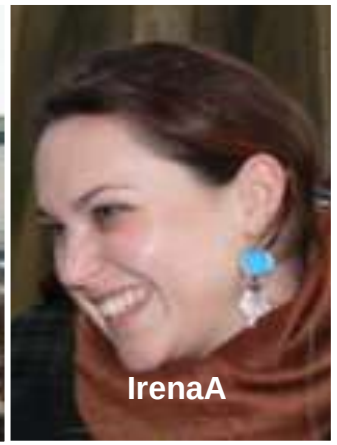
Relman lab:	Boschwitz *et al.* (1997) *JID* 176:678	Human MoDCs
Guiso lab:	Njamkempo *et al.* (2000) *J. Cell. Physiol.* 183:91	Human Monocytes
Lewis lab:	Bagley *et al.* (2002): *J. Leukoc. Biol.* 72:962	Human MoDCs
Mills lab:	Ross *et al.* (2004) *Infect. Immun.* 72:1568	Mouse BMDCs
Mills lab:	Boyd *et al.* (2005) *J. Immunol.* 175: 730	Mouse BMDCs
Ausiello lab:	Spensieri *et al.* (2006) *Infect. Immun.* 74:2831	Human MoDC
Mills lab:	Hickey *et al.* (2008) *J. Leukoc. Biol.* 84:234	Mouse BMDCs
Ausiello lab:	Fedele *et al.* (2010) *PLoS One.* 5(1): e8734	Human MoDC
Sebo lab:	Adkins et al. unpublished	BMDC and Human MoDC

cAMP signaling of ACT promotes migration of DC

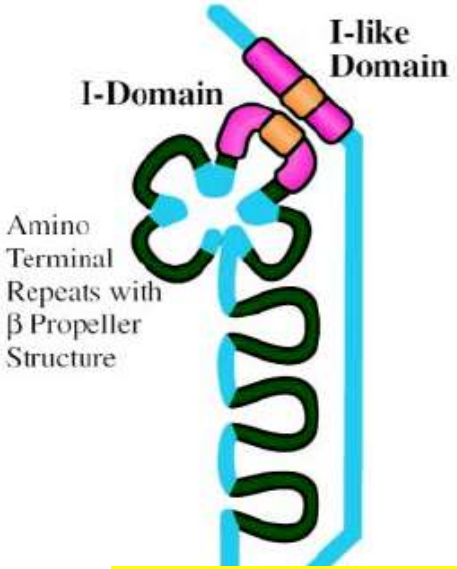
Outmigration of tolerogenic DC from mucosa into lymph nodes to hamper antigen-specific T cell responses???







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

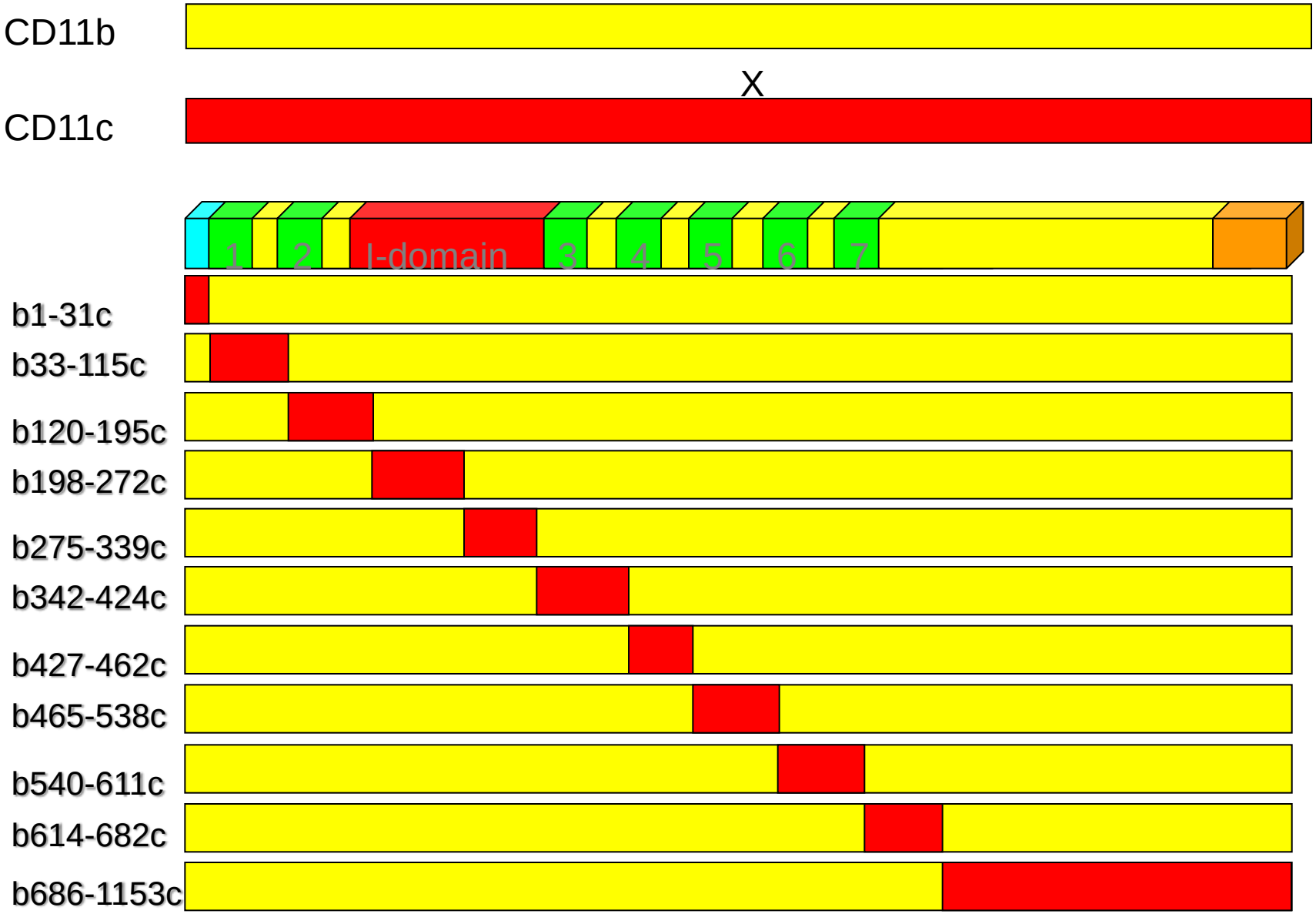
ACT first recognizes N-linked glycans of CD11b/CD18

Morova et al. (2008) PNAS 105, 5355

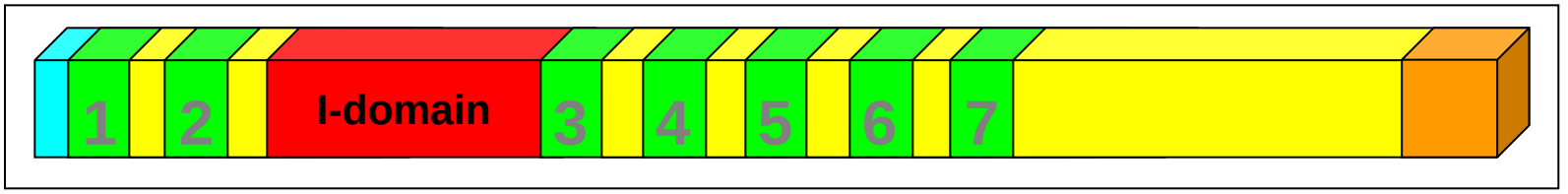
Guermonprez et al. 2001, J Exp. Med.

Selectivity of ACT for the given $\alpha_M\beta_2$ integrin is, however, dictated by a CD11b-specific segment …

Mapping ACT binding site using CD11b/CD11c chimaeras



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



CD11b-YFP

X

CD11c-YFP

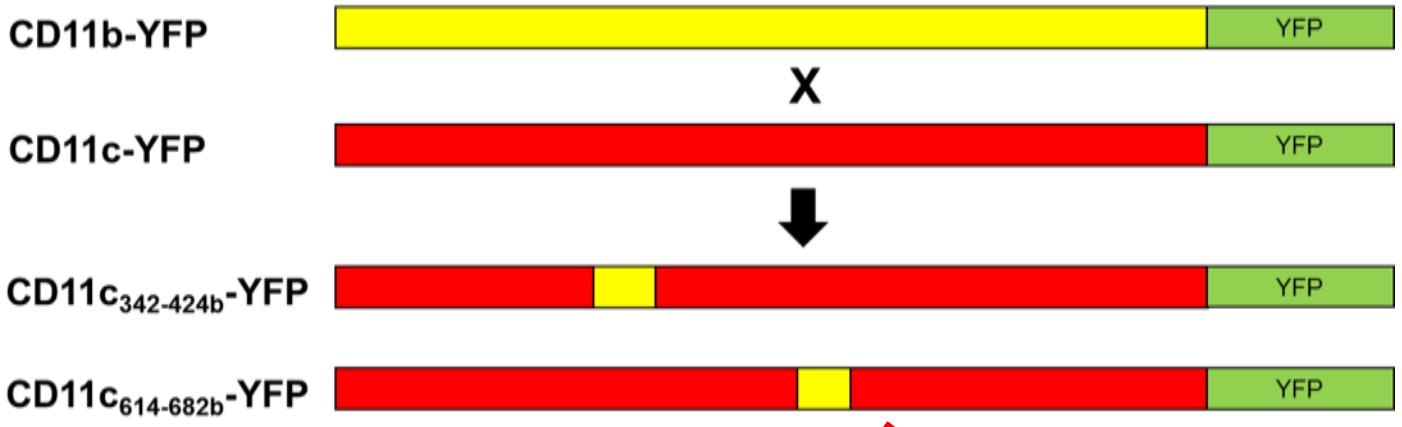
CD11c342-424b-YFP

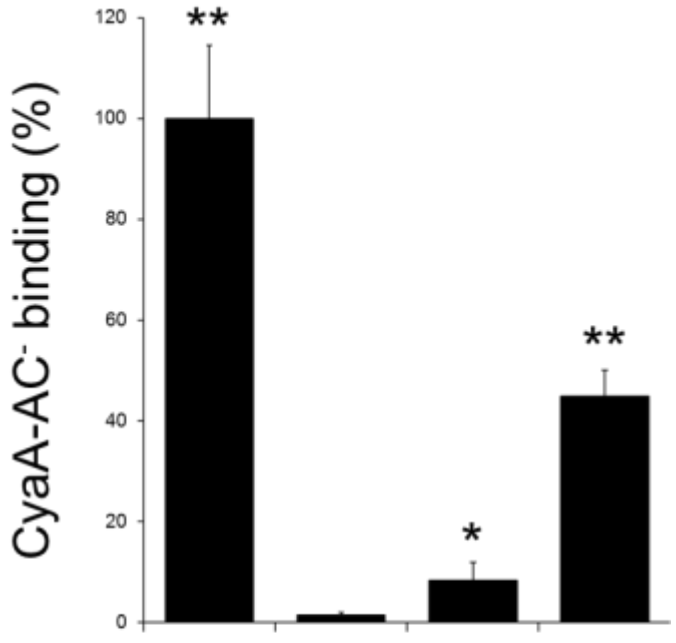
342 424

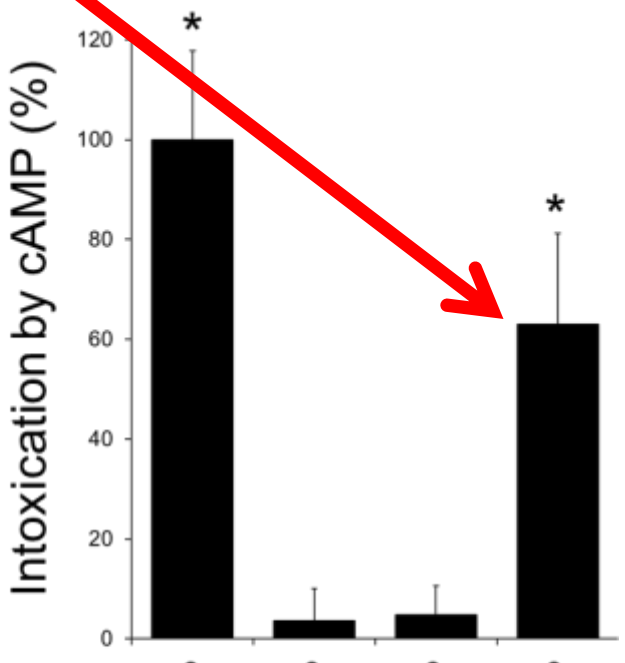
CD11c614-682b-YFP

614 682

The CD11c integrin with ‘transplanted’ residues 614-682 of CD11b productively binds CyaA



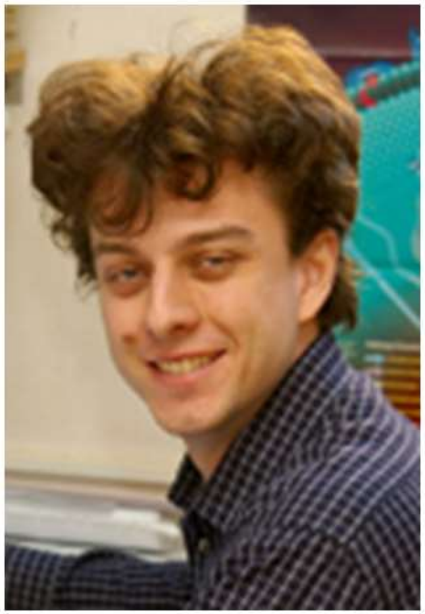


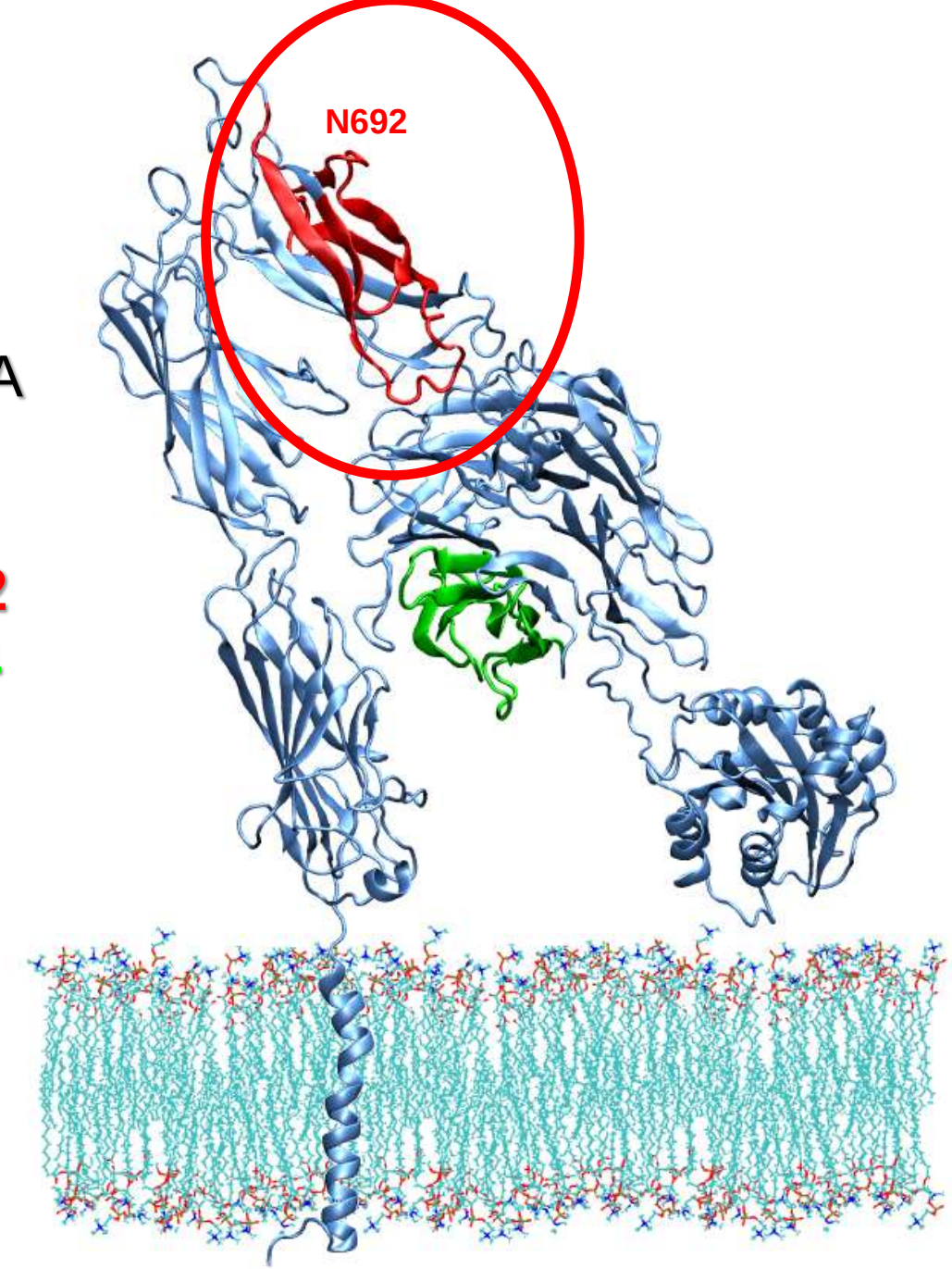


Proteinaceous segments Specifically involved in CyaA binding:

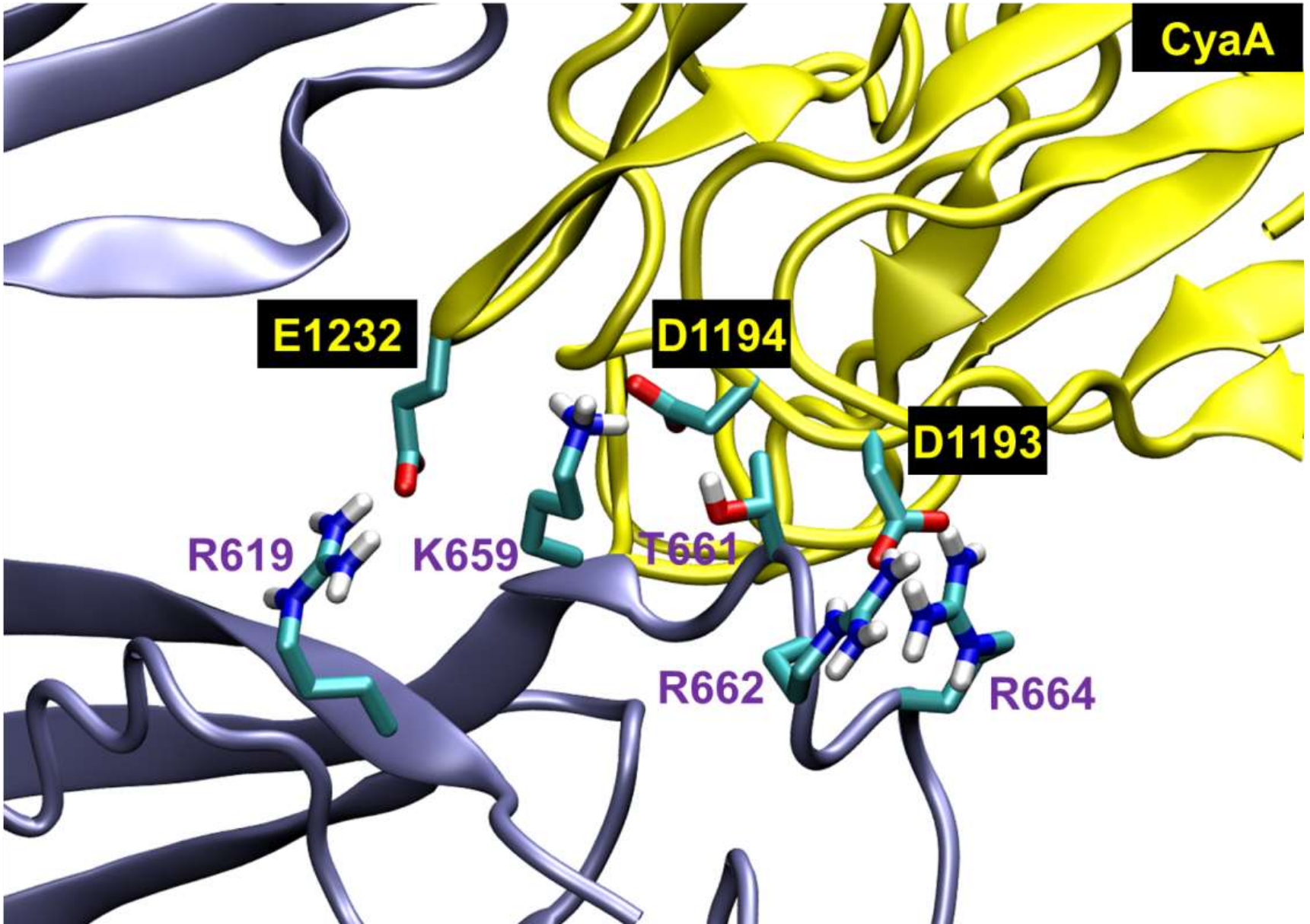
CD11b – residues 614-682

CD11b - residues 342-424



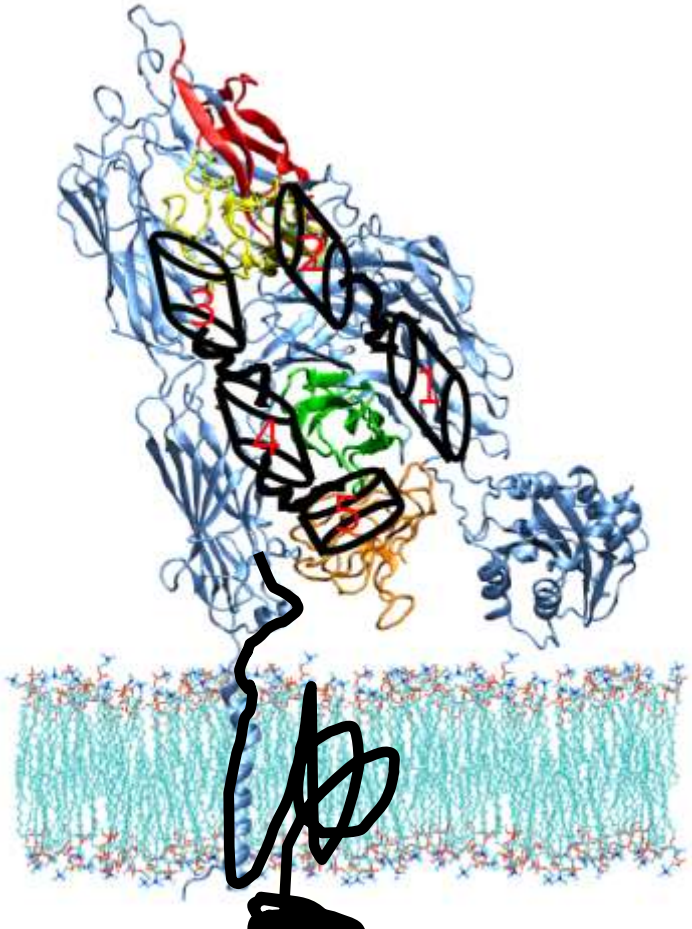


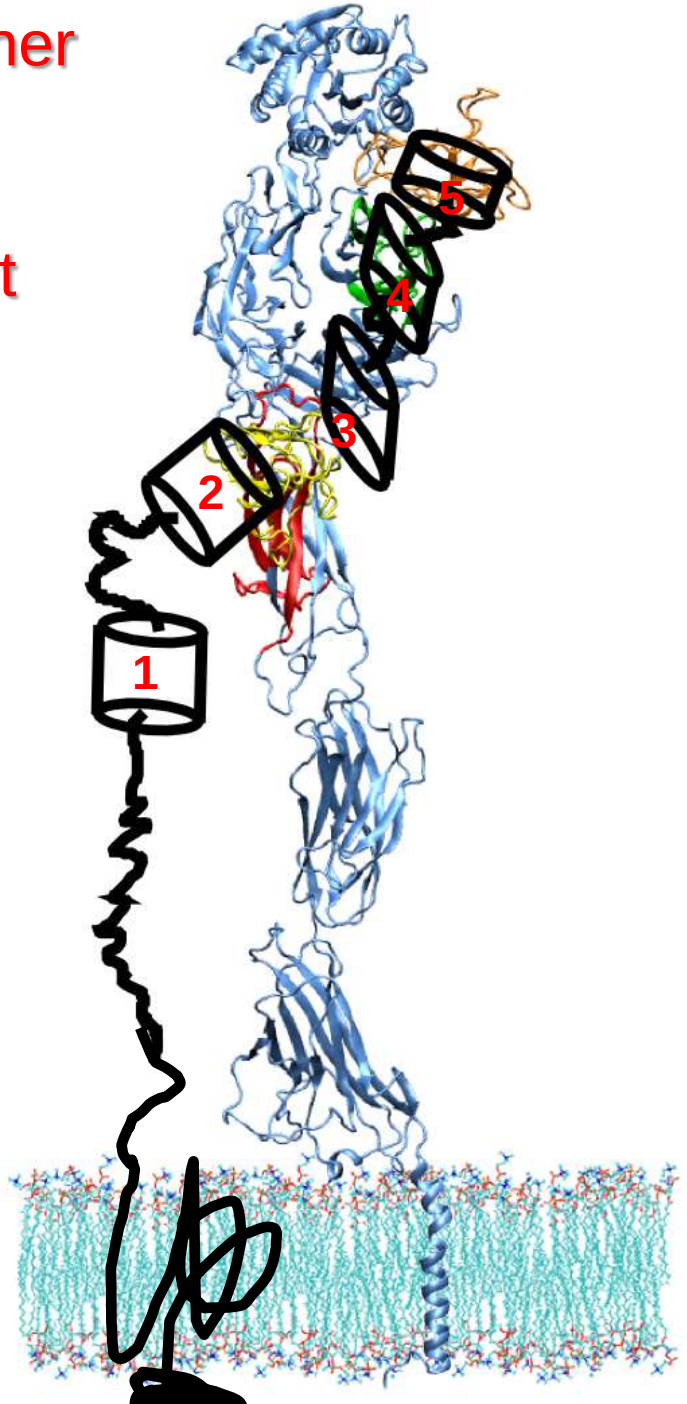
Recognition of CD11b by CyaA depends on electrostatic interaction of oppositely charged toxin and integrin segments



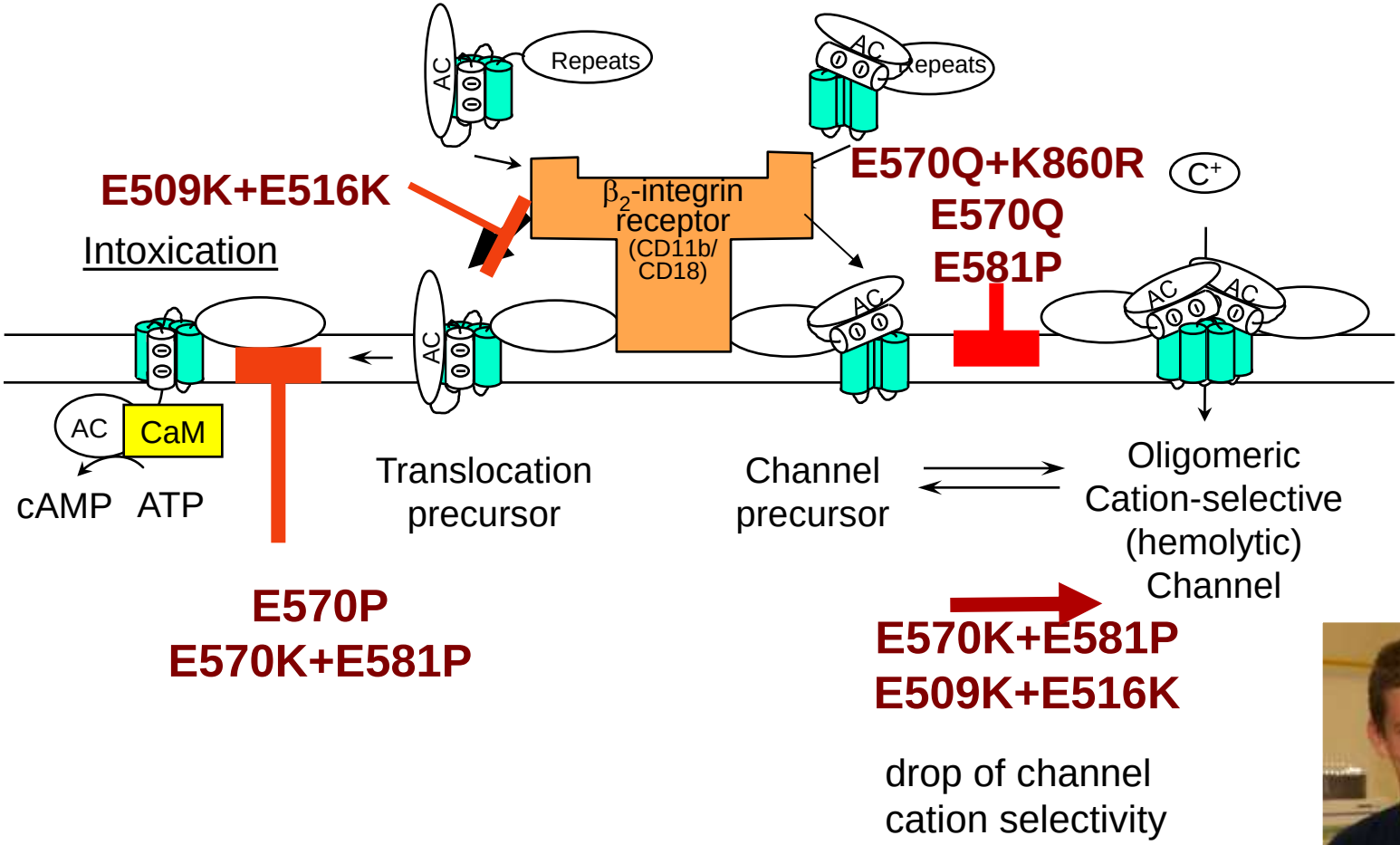
CyaA RTX domain – binds with 3 x higher affinity to ‘closed’ CD11b

Subversive toxin binding occurs without integrin Syk/Src signaling activation…



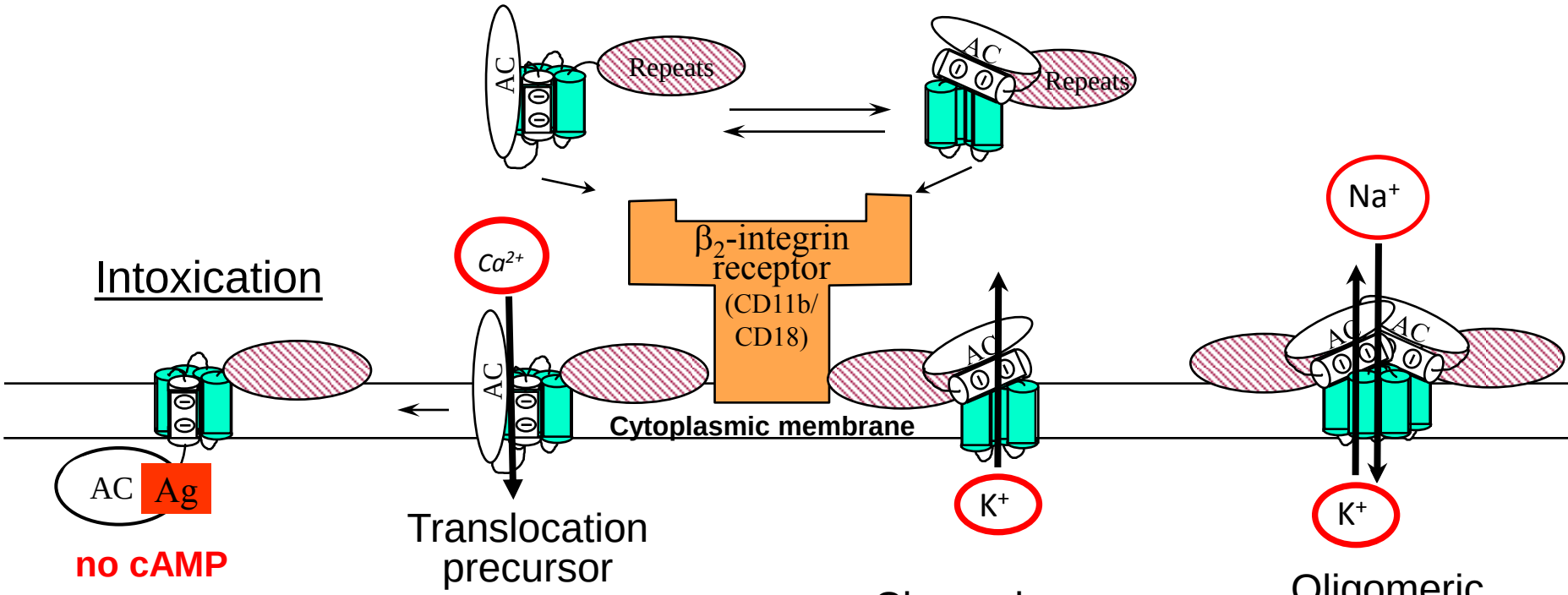


The toolbox: **A panel of mutations characterized that block ACT activity at each individual step of toxin action**



Basler *et al.*, (2007) *J. Biol. Chem.* **282**, 12419

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

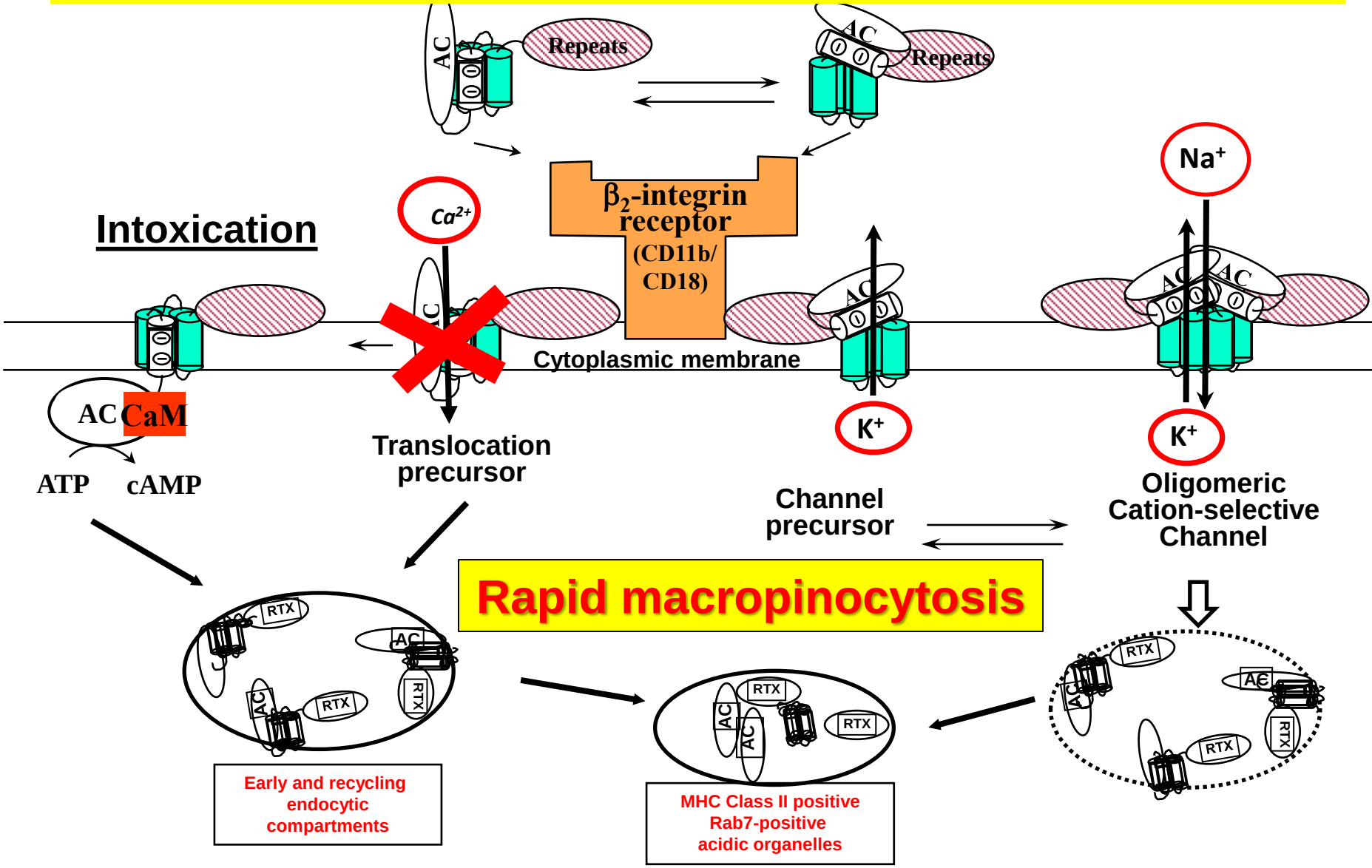


Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Osickova *et al.* (2010) *Mol. Microbiol. 75:15450-1562*

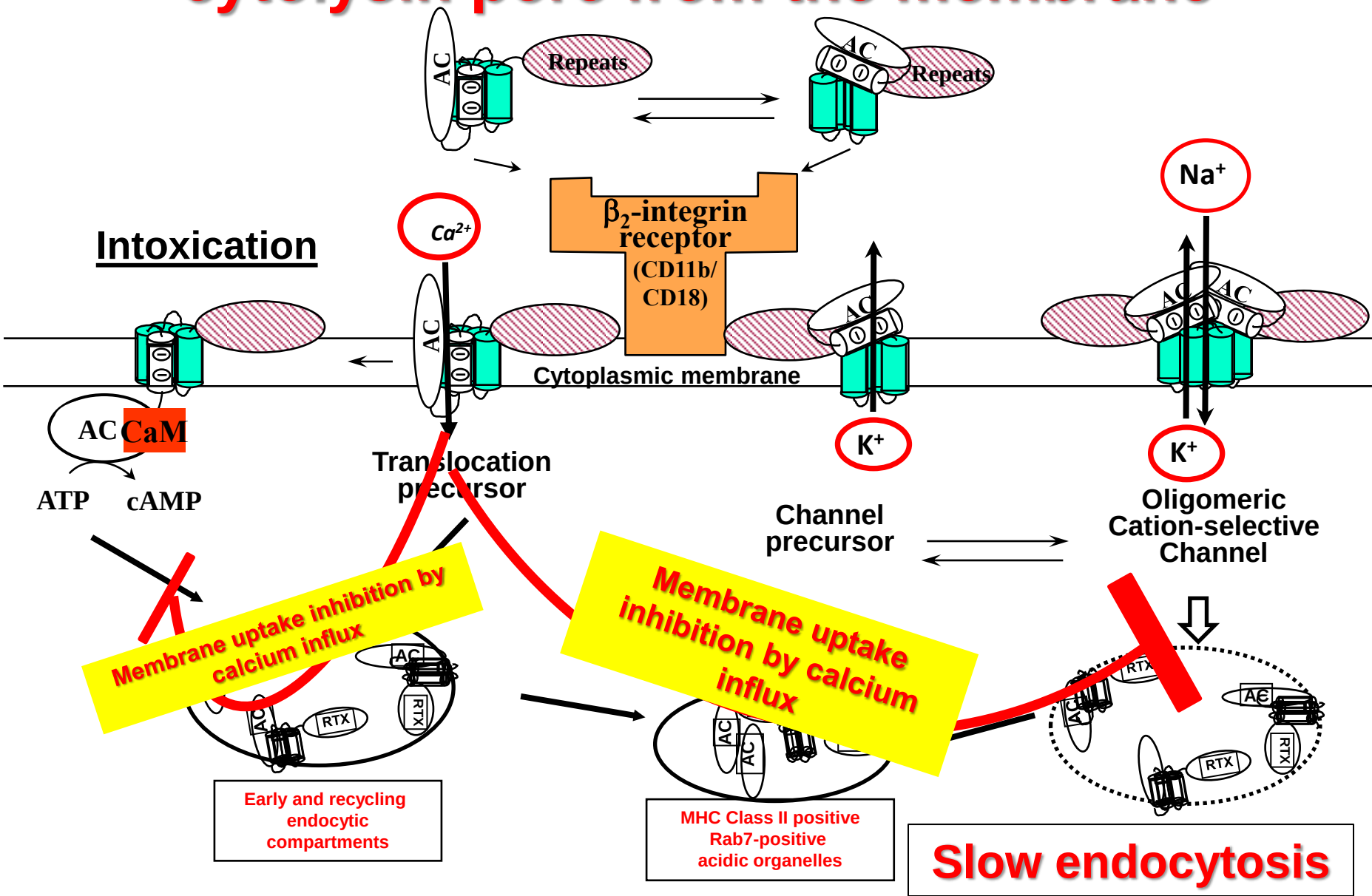
Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*

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

Toxoids unable to cause calcium influx are rapidly endocytosed with the receptor



Calcium influx impairs removal od the cytolysin pore from the membrane



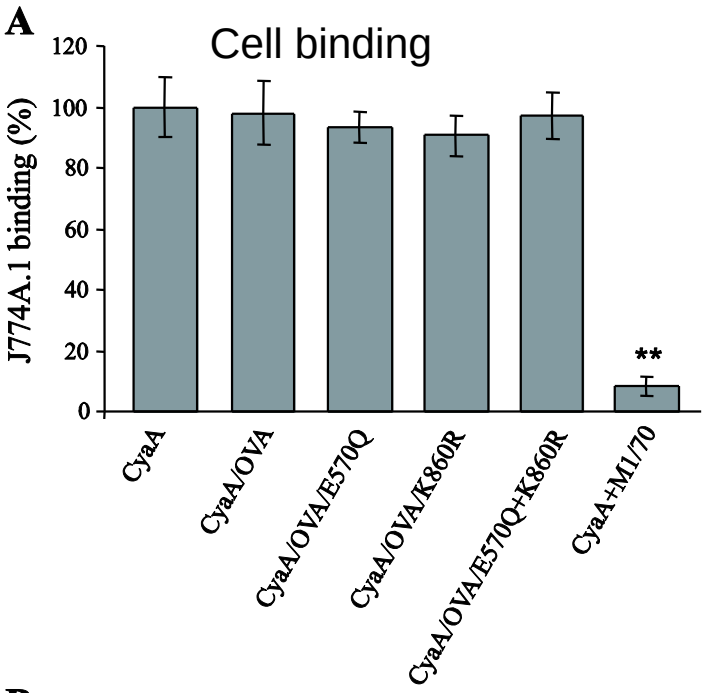
The sophistication of toxin action:

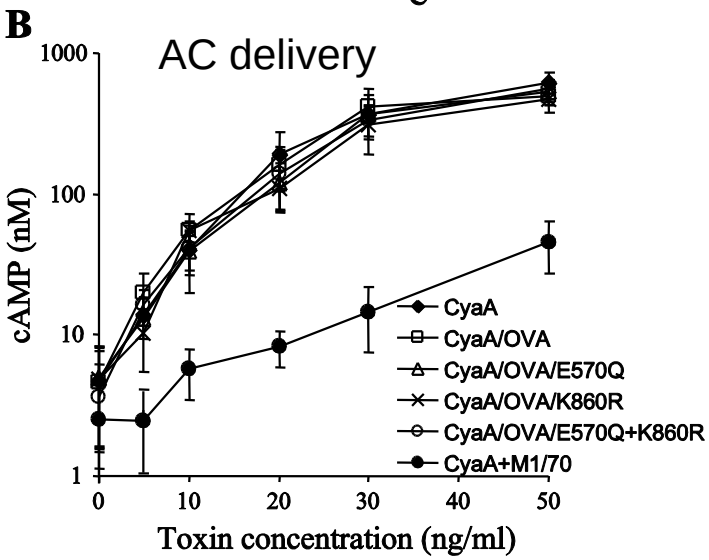
- By allowing calcium to enter cells, CyaA initiates a positive feedback loop to protract its persistence in cell membrane to cause extensive cell permeabilization and potassium efflux:

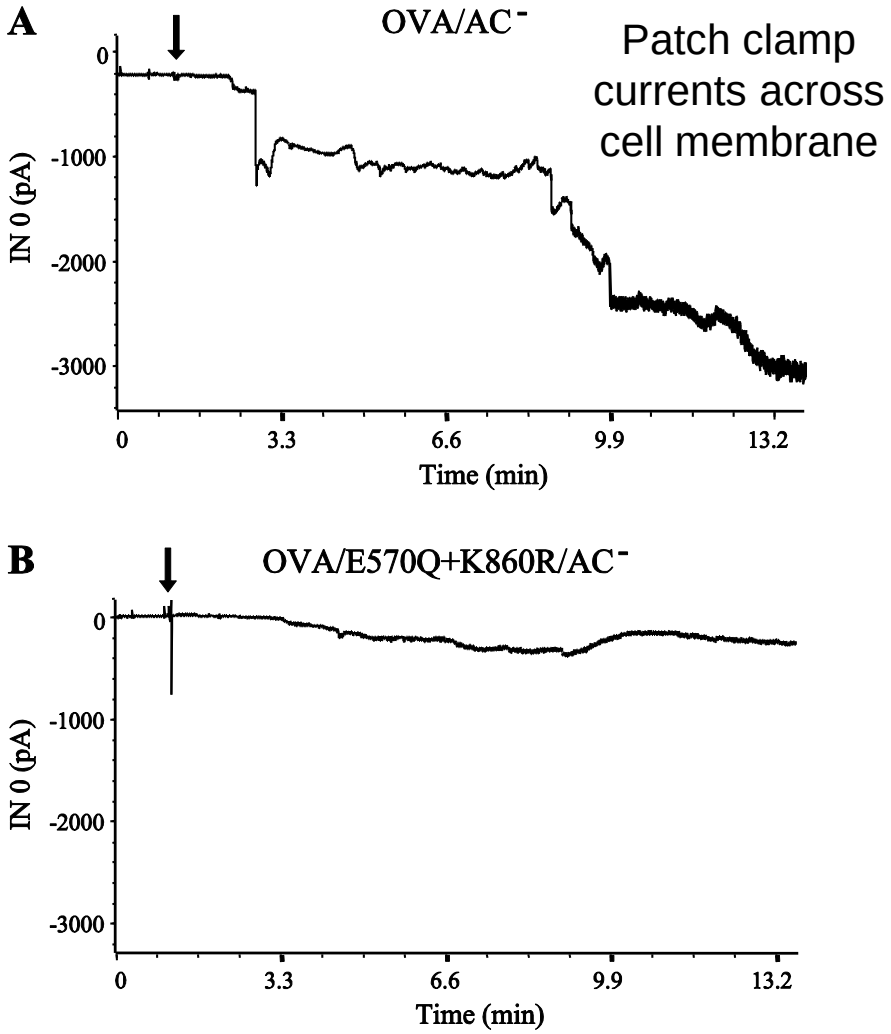
- **The more potassium flows out, the slower is the pore removed (endocytosed) by clathrin-dependent uptake and = the more potassium leaks out…**

Fiser *et al.*: PLoS Pathogens 2012;8(4):e1002580

AC domain is not translocated across the CyaA pore



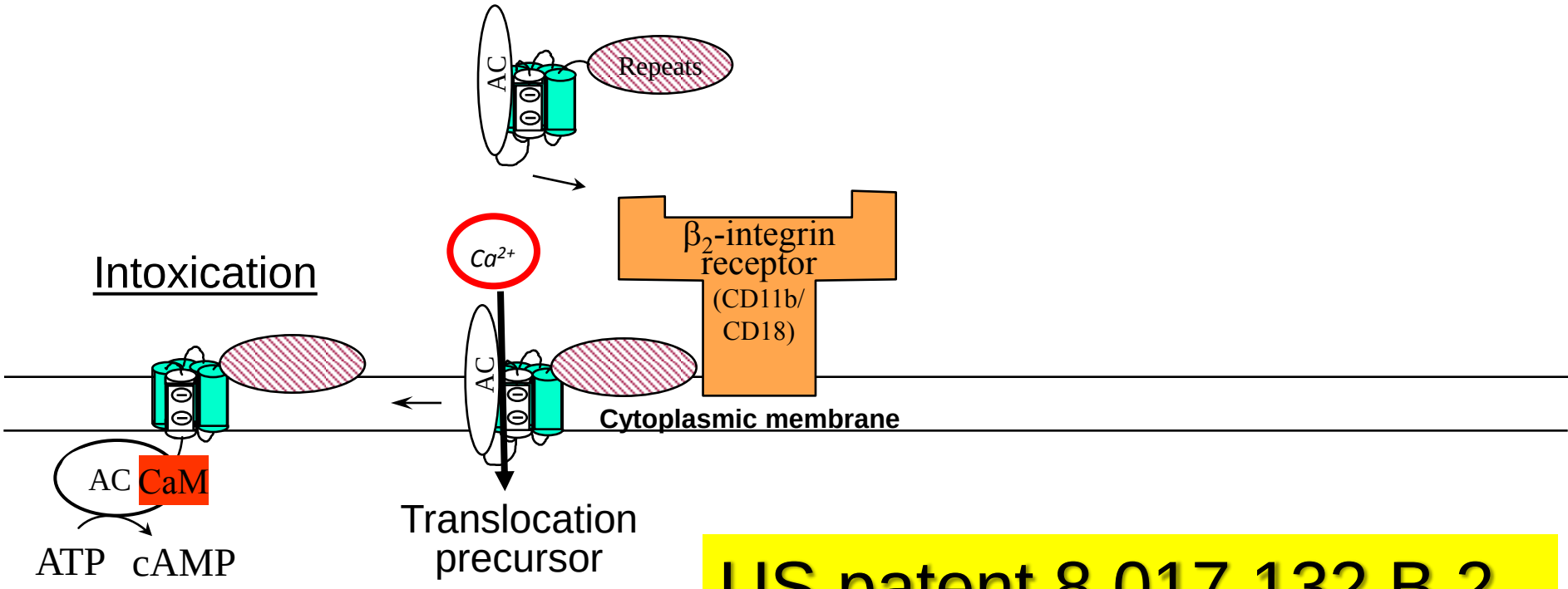




The E570Q+K860R toxin delivers the AC domain into cells without permabilizing he cell membrane

Osickova *et al.* (2010) *Mol. Microbiol. 75:15450-1562*

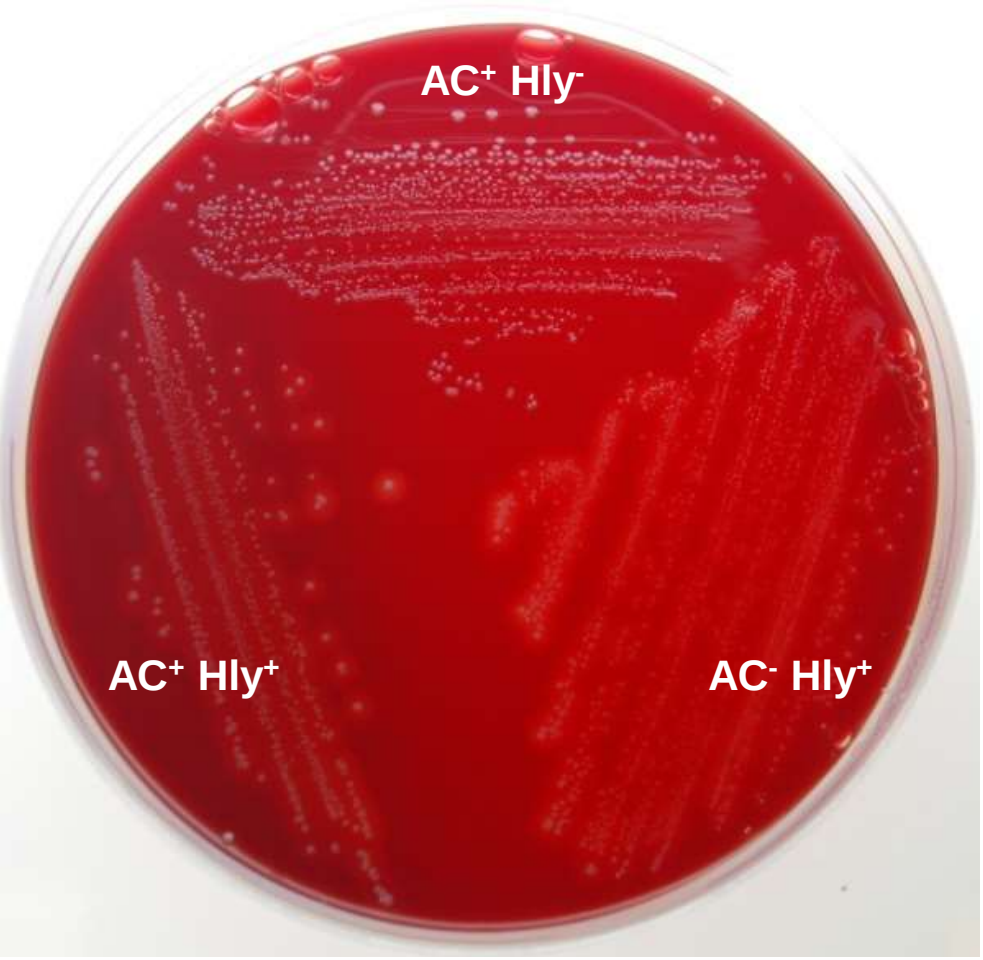
So, this appears to be real…



US patent 8,017,132 B.2

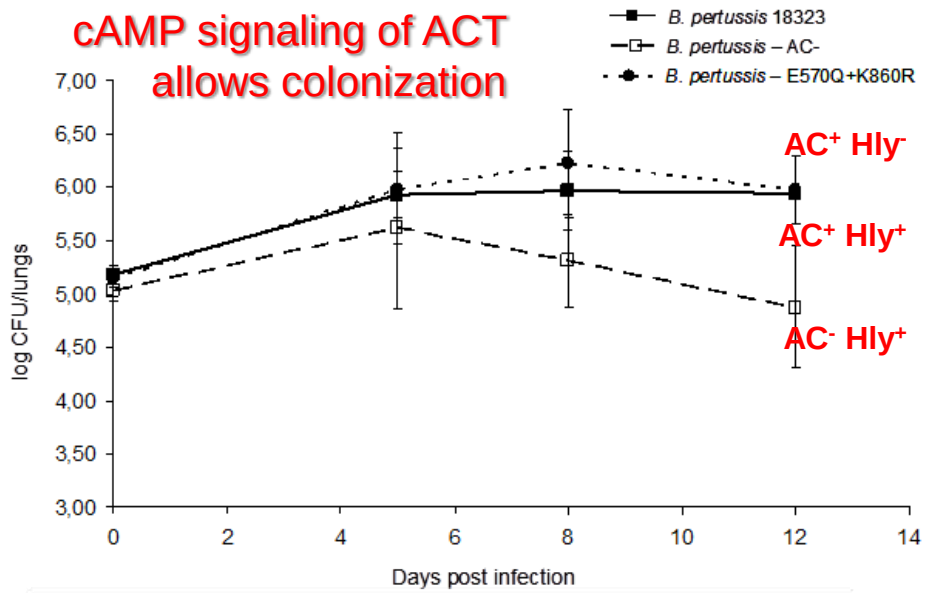
Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Basler et al., (2007) J. Biol. Chem. 282, 12419
Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*

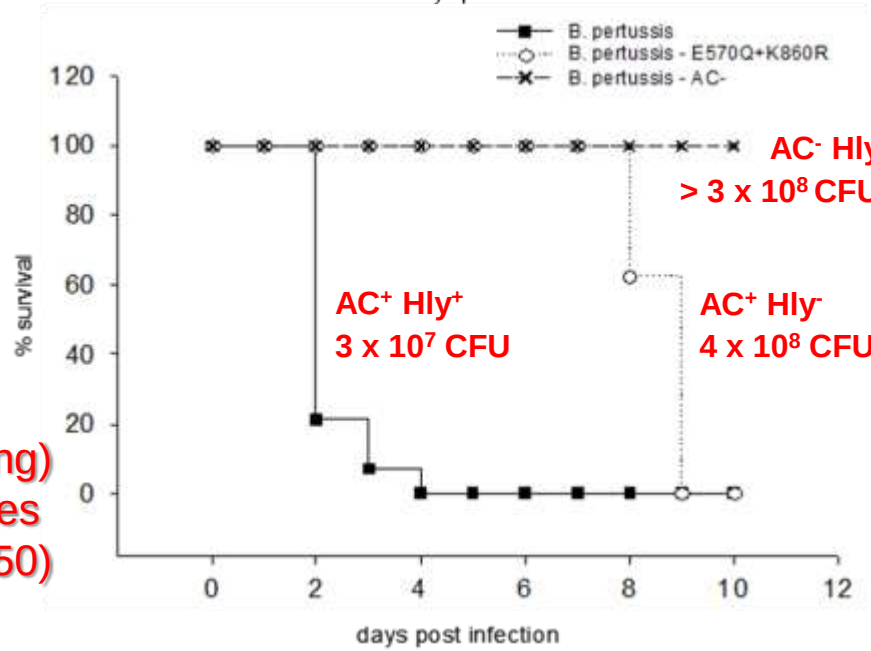
AC⁻ *B. pertussis* is avirulent and pore-forming (hemolytic) activity contributes virulence

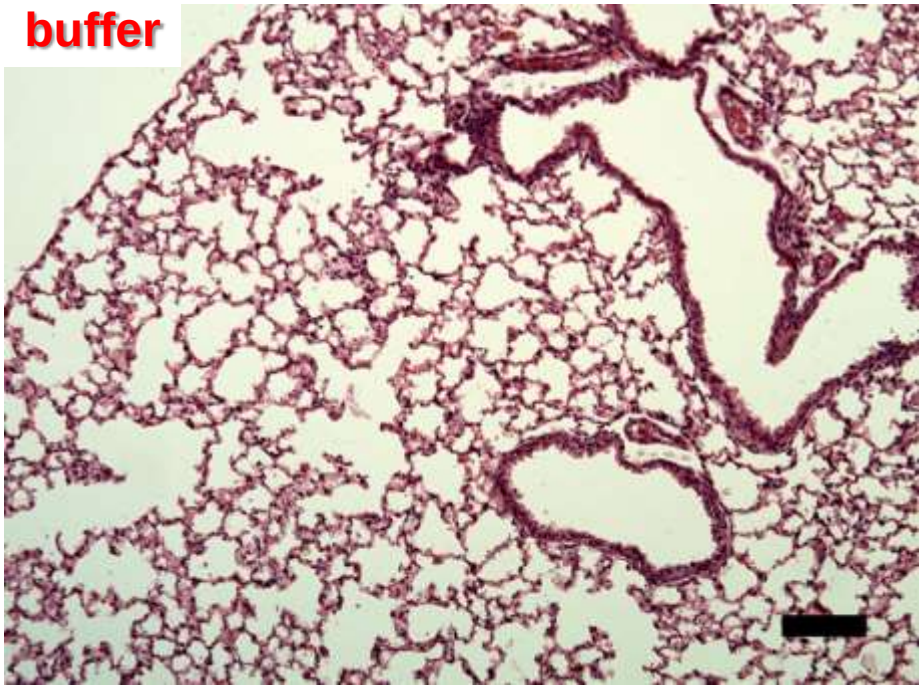


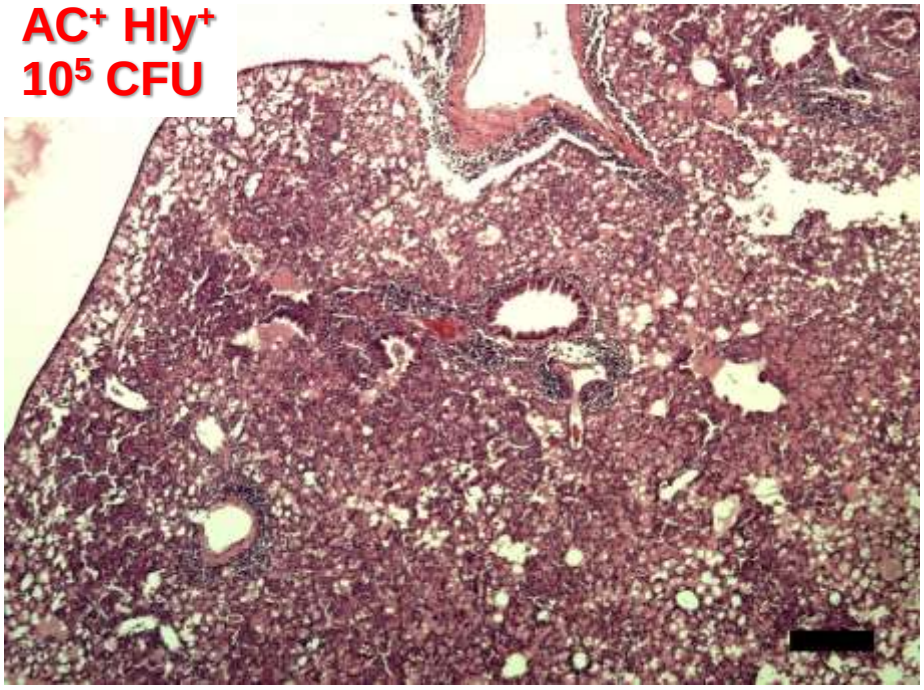
Hemolytic (pore-forming) activity contributes to pathology (LD50)

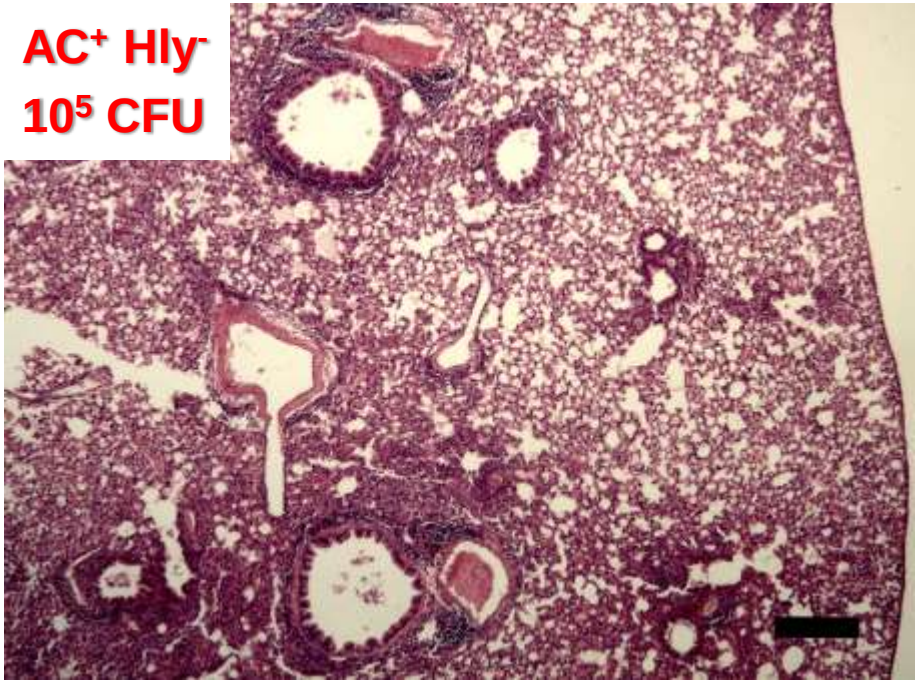
cAMP signaling of ACT allows colonization

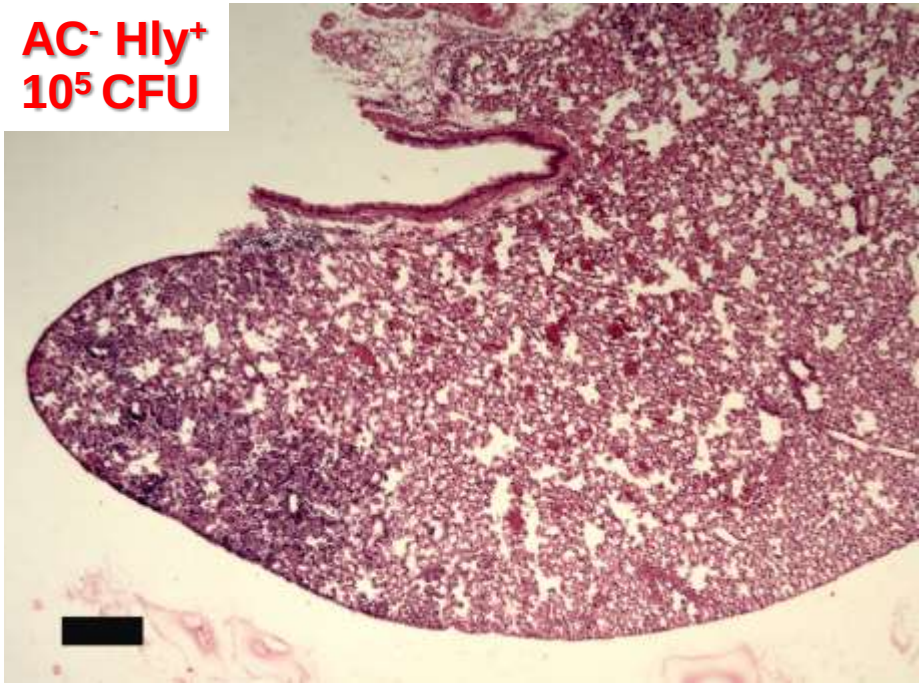





buffer


AC+ Hly+
10^5 CFU


AC+ Hly-
10^5 CFU


AC- Hly+
10^5 CFU

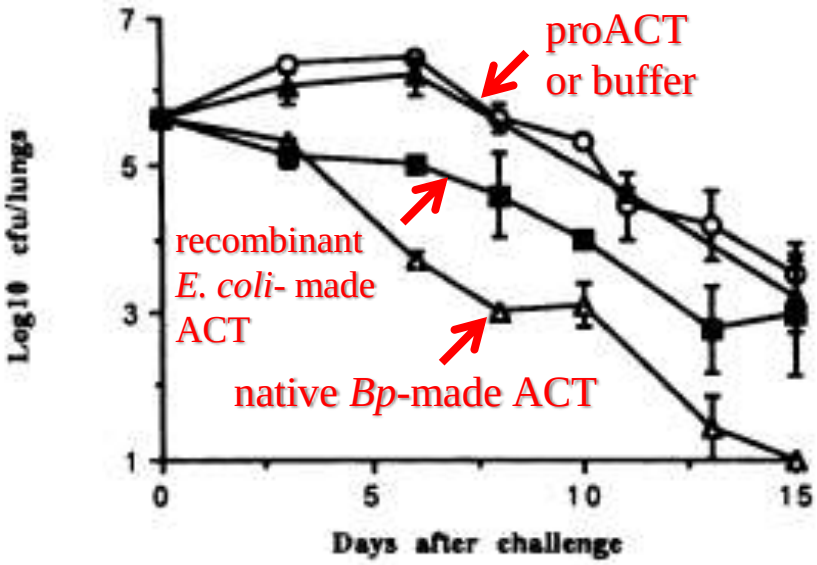
Not suprisingly, hence, ACT is a protective antigen

INFECTION AND IMMUNITY, Sept. 1993, p. 3583-3589
0019-9567/93/093583-07$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 61, No. 9

CyaC-Mediated Activation Is Important Not Only for Toxic but Also for Protective Activities of *Bordetella pertussis* Adenylate Cyclase-Hemolysin

FOTINI BETSOU,[1] PETER ŠEBO,[2] AND NICOLE GUISO[1]*
Unité de Bactériologie Moléculaire et Médicale[1] and Unité de Biochimie des Régulations Cellulaires,[2] Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

Received 8 April 1993/Returned for modification 7 May 1993/Accepted 1 June 1993



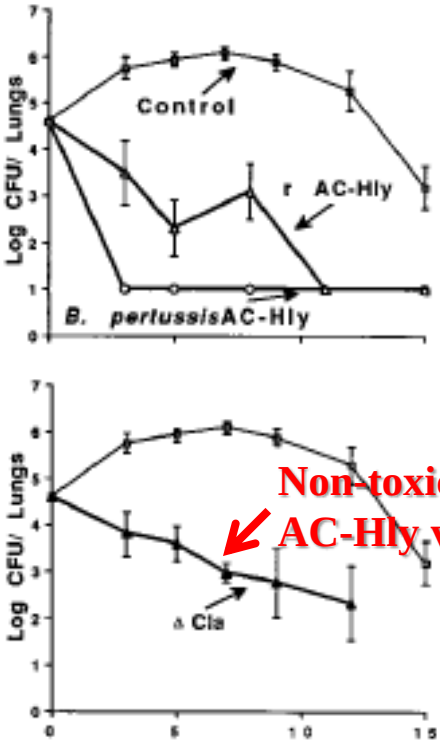
(at that time ACT samples contained LPS)

INFECTION AND IMMUNITY, Sept. 1995, p. 3309-3315
0019-9567/95/$04.00+0
Copyright © 1995, American Society for Microbiology
Vol. 63, No. 9

The C-Terminal Domain Is Essential for Protective Activity of the *Bordetella pertussis* Adenylate Cyclase-Hemolysin

FOTINI BETSOU,[1] PETER SEBO,[2]† AND NICOLE GUISO[1]*
Unité de Bactériologie Moléculaire et Médicale[1] and Unité de Biochimie des Régulations Cellulaires,[2] Institut Pasteur, 75724 Paris Cedex 15, France

Received 1995



Addition of CyaA-AC$^-$ improves performance of the aP vaccine

INFECTION AND IMMUNITY, Dec. 2006, p. 6797–6805 Vol. 74, No. 12
0019-9567/06/$08.00+0 doi:10.1128/IAI.01104-06

Effect of Different Forms of Adenylate Cyclase Toxin of *Bordetella pertussis* on Protection Afforded by an Acellular Pertussis Vaccine in a Murine Model[▽]

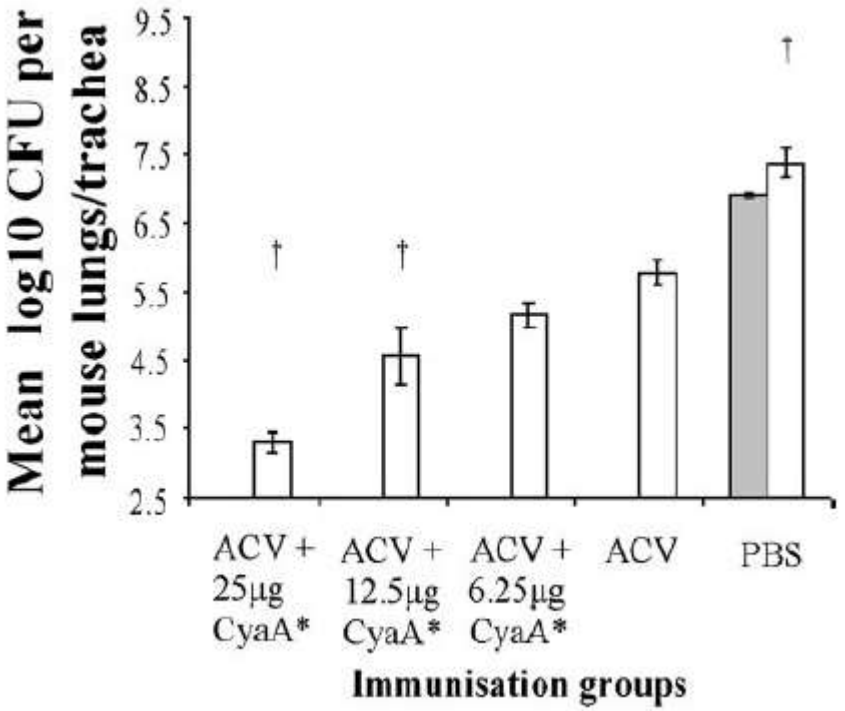
Gordon Y. C. Cheung,[1] Dorothy Xing,[2] Sandra Prior,[2] Michael J. Corbel,[2] Roger Parton,[1] and John G. Coote[1]*

Division of Infection and Immunity, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow,[1] and Division of Bacteriology, National Institute of Biological Standards and Control, South Mimms, Hertfordshire,[2] United Kingdom

Received 14 July 2006/Accepted 12 September 2006

Four recombinant forms of the cell-invasive adenylate cyclase toxin (CyaA) of *Bordetella pertussis* were compared for the ability to enhance protection against *B. pertussis* in mice when coadministered with an acellular pertussis vaccine (ACV). The four forms were as follows: fully functional CyaA, a CyaA form lacking adenylate cyclase enzymatic activity (CyaA*), and the nonacylated forms of these toxins, i.e., proCyaA and proCyaA*, respectively. None of these forms alone conferred significant ($P > 0.05$) protection against *B. pertussis* in a murine intranasal challenge model. Mice immunized with ACV alone showed significant ($P < 0.05$) reductions in bacterial numbers in the lungs after intranasal challenge compared with those for control mice. When administered with ACV, both CyaA and CyaA* further reduced bacterial numbers in the lungs of mice after intranasal challenge compared with those for ACV-immunized mice, but the enhanced protection was only significant ($P < 0.05$) with CyaA*. Coadministration of CyaA* with ACV caused a significant ($P < 0.05$) increase in immunoglobulin G2a antibody levels against pertactin compared with those in mice immunized with ACV alone. Spleen cells from mice immunized with ACV plus CyaA* secreted larger amounts of interleukin-5 (IL-5), IL-6, gamma interferon (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) than did cells from mice immunized with ACV plus CyaA or ACV alone after stimulation in vitro with a mixture of *B. pertussis* antigens. Spleen cells from mice immunized with ACV plus CyaA* also secreted larger amounts of IFN-γ and GM-CSF than did cells from mice immunized with CyaA* alone after stimulation in vitro with CyaA*. Macrophages from mice immunized with ACV plus CyaA* produced significantly ($P < 0.05$) higher levels of nitric oxide than did macrophages from mice immunized with CyaA* alone, ACV alone, or ACV plus CyaA after stimulation in vitro with a mixture of *B. pertussis* antigens or heat-killed *B. pertussis* cells. These data suggest that the enhancement of protection provided by CyaA* was due to an augmentation of both Th1 and Th2 immune responses to *B. pertussis* antigens.

one-eighth of human dose of DTaP/ACV
(Infanrix, GSK) **+ CyaA-AC$^-$** 2 x i.p.
challenged with 4 x 10^6 B. pertussis 18.323 i.n.



Highly purified CyaA-AC$^-$ protects on its own

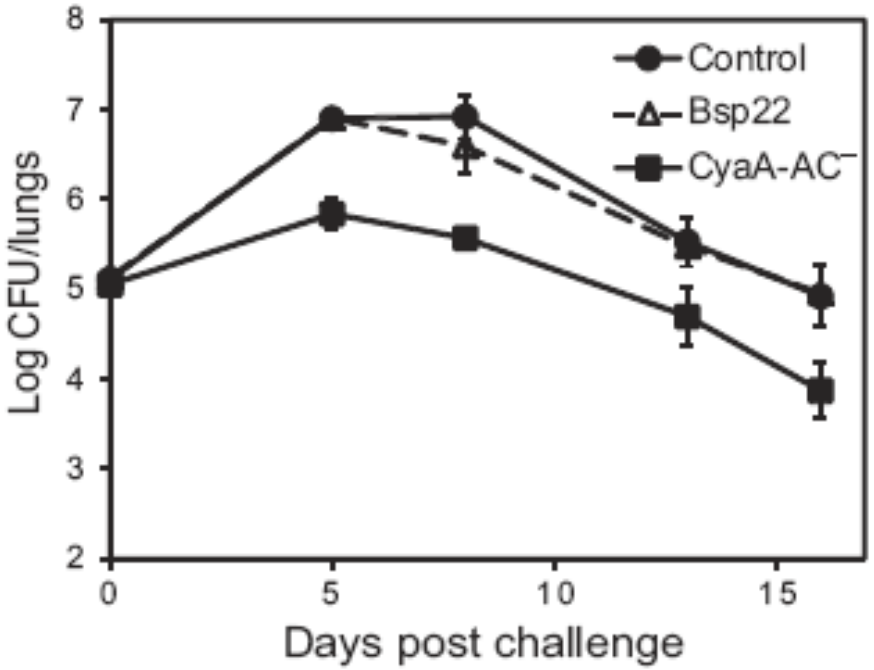
Infection and Immunity **81:** 2761–2767 (2013)

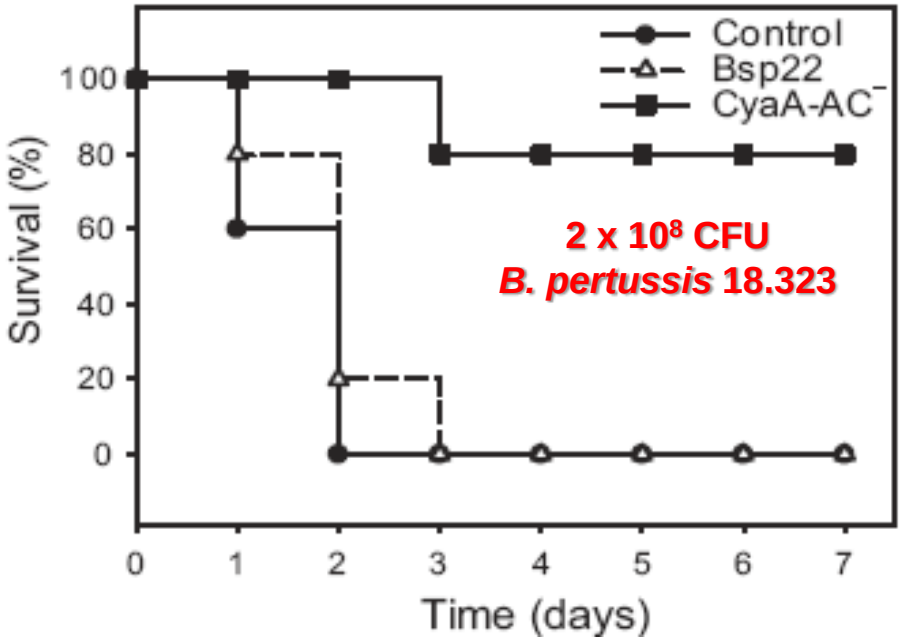
The *Bordetella pertussis* Type III Secretion System Tip Complex Protein Bsp22 Is Not a Protective Antigen and Fails To Elicit Serum Antibody Responses during Infection of Humans and Mice

Rodrigo Villarino Romero,[a] Ilona Bibova,[a] Ondrej Cerny,[a] Branislav Vecerek,[a] Tomas Wald,[a] Oldrich Benada,[a] Jana Zavadilova,[b] Radim Osicka,[a] Peter Sebo[a]

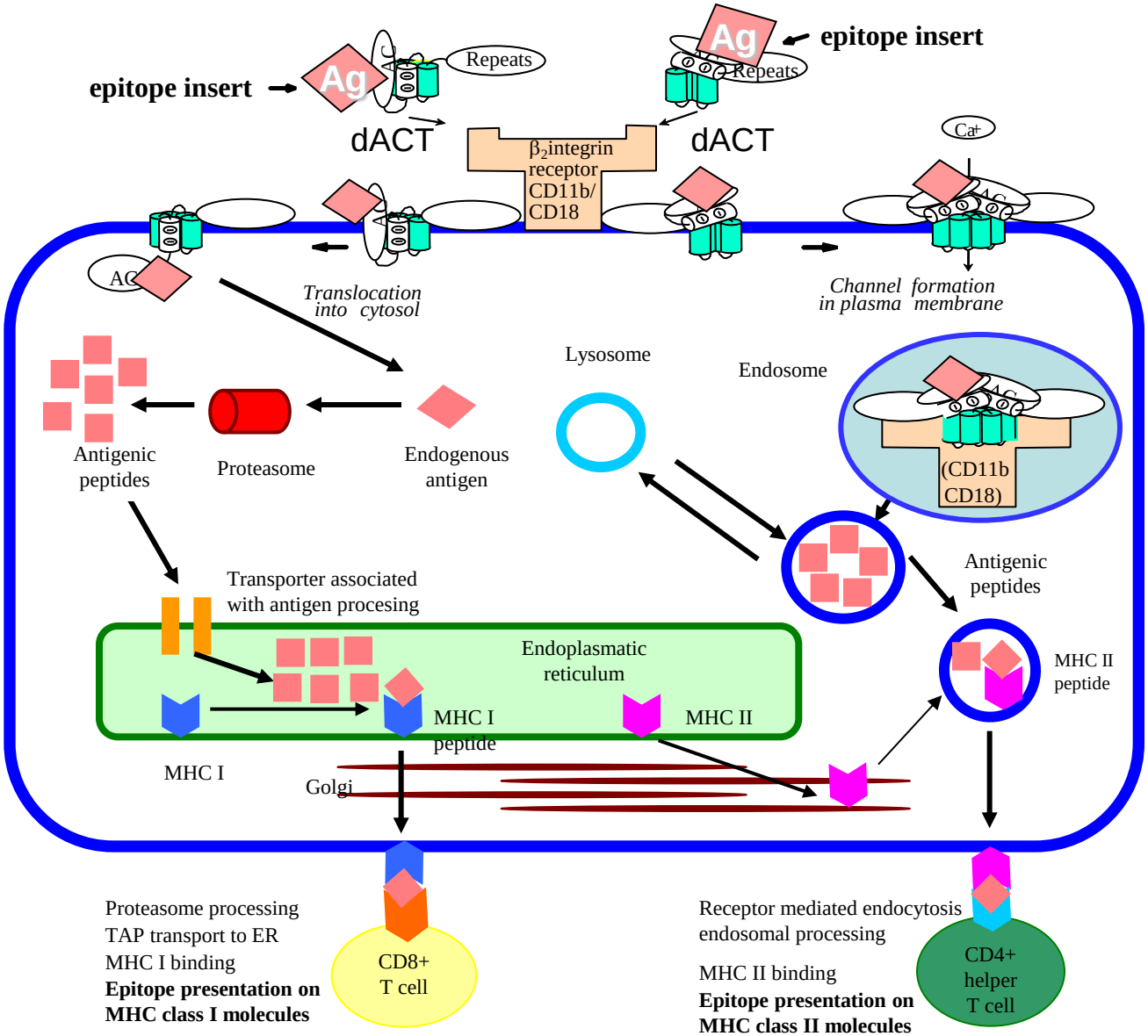
Institute of Microbiology of the ASCR, Prague, Czech Republic[a]; National Institute of Public Health, Prague, Czech Republic[b]

Rodrigo Ilona





dACT as a novel antigen delivery tool



September 2012 - Genticel S.A. completed Phase I clinical trial for HPV16/18-induced cervical carcinoma

Using a cGMP batch of the adenylate cyclase (CyaA-AC$^-$) toxoid for delivery of HPV E7 antigen as immunotherapeutic vaccine

safe, immunogenic, inducing CD8$^+$ CTLs and HPV 16/18 virus load reduction demonstrated

…Heading for phase II trial = will be of interest to see pertussis incidence in CyaA-E7 toxoid treated woman…

In conclusion:

The pore-forming (hemolytic) activity of ACT is important for *B. pertussis* virulence, contributing to inflammation that will eventually help to clear the infection (in mice)

The **cAMP signaling of ACT,**

however, prevails and

knocks down the innate immunity

and

dampens the adaptive immune response

in order

to enable host colonization

Explaining why is it so important
to add the AC toxoid
into the aP vaccine

if we are serious about

breaking the vicious circle
of epidemic whooping cough
spread in the most developed
countries …

Sebolab = a Confederation of PIs…

Radim Osička – CR3 receptor interactions of ACT

Jiří Mašín – role of pore-forming activity of ACT

Láďa Bumba – folding and secretion of ACT

Branko Večerek – sRNA regulation in *Bordetella*

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External collaborations:

Institut Pasteur:

teams:

Nicole Guiso

Claude Leclerc

University of Virginia

Erik L. Hewlett

Trinity College

Kingston Mills

Aisling Dunne

Institute of Microbiology

Lída Tučková

Marek Kovář

and their teams

University Wurzburg

Roland Benz

and his team

Bernhard Nocht Institut:

Thomas Jacobs

Susanne Tartz

MH Hannover

Ingo Just

Harald Genth

VLA Surrey

Martin Vordemeier