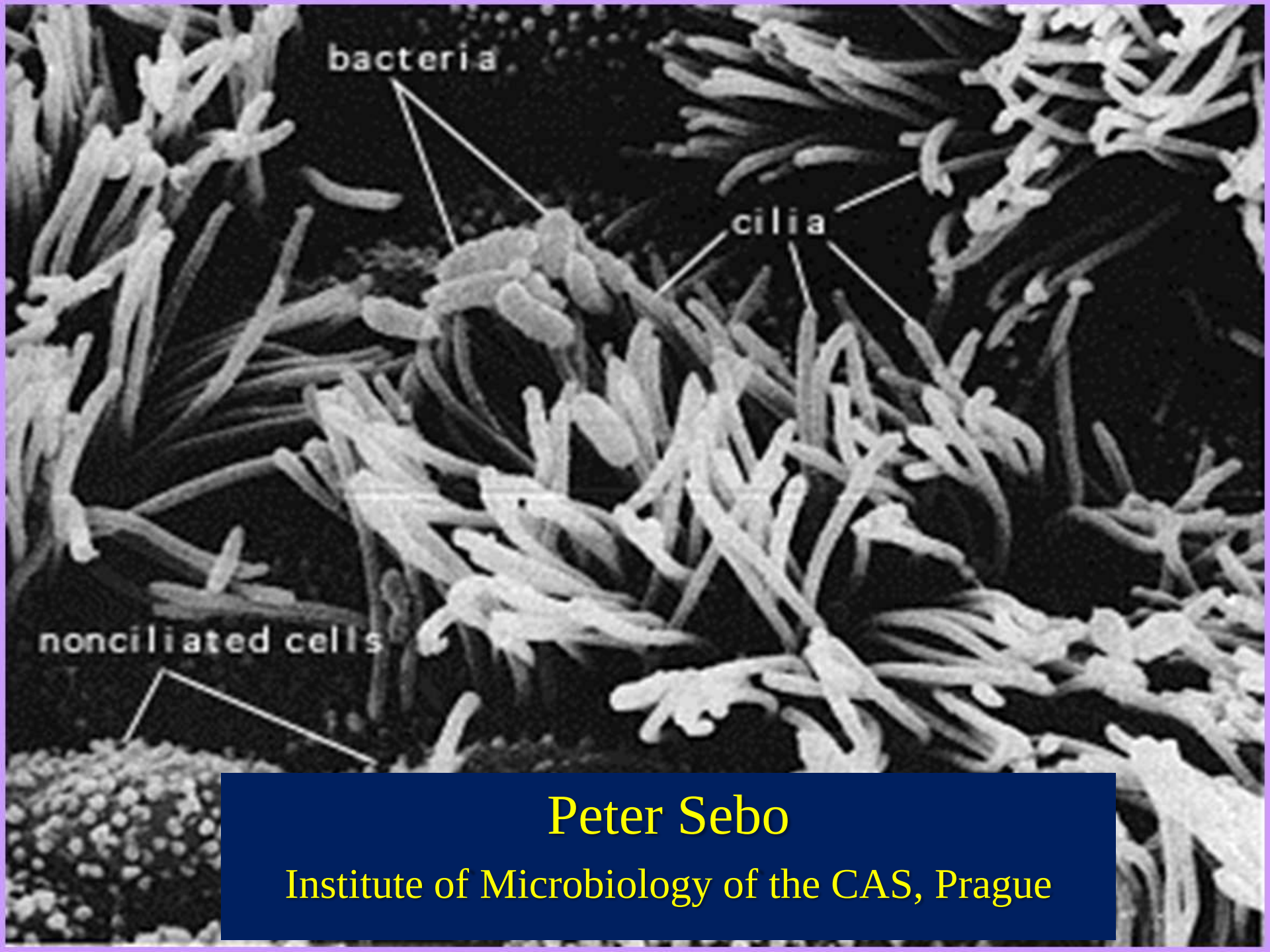


Peter Sebo

Institute of Microbiology of the CAS, Prague

Baltimore 2010 / Dublin 2013 / Annecy 2015:

thanks to

‘**democracy**’

and

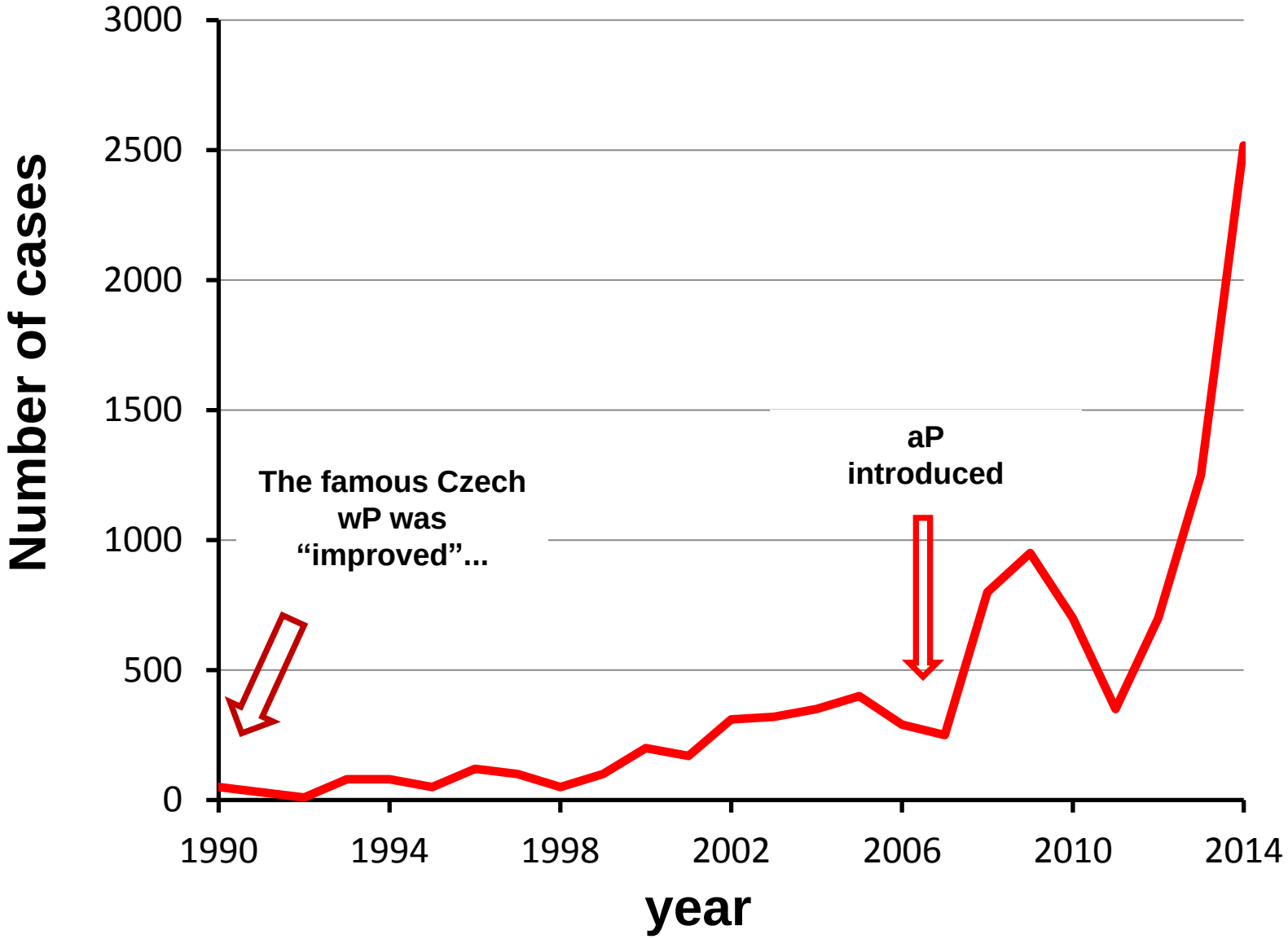
acellular vaccines

whooping cough is back

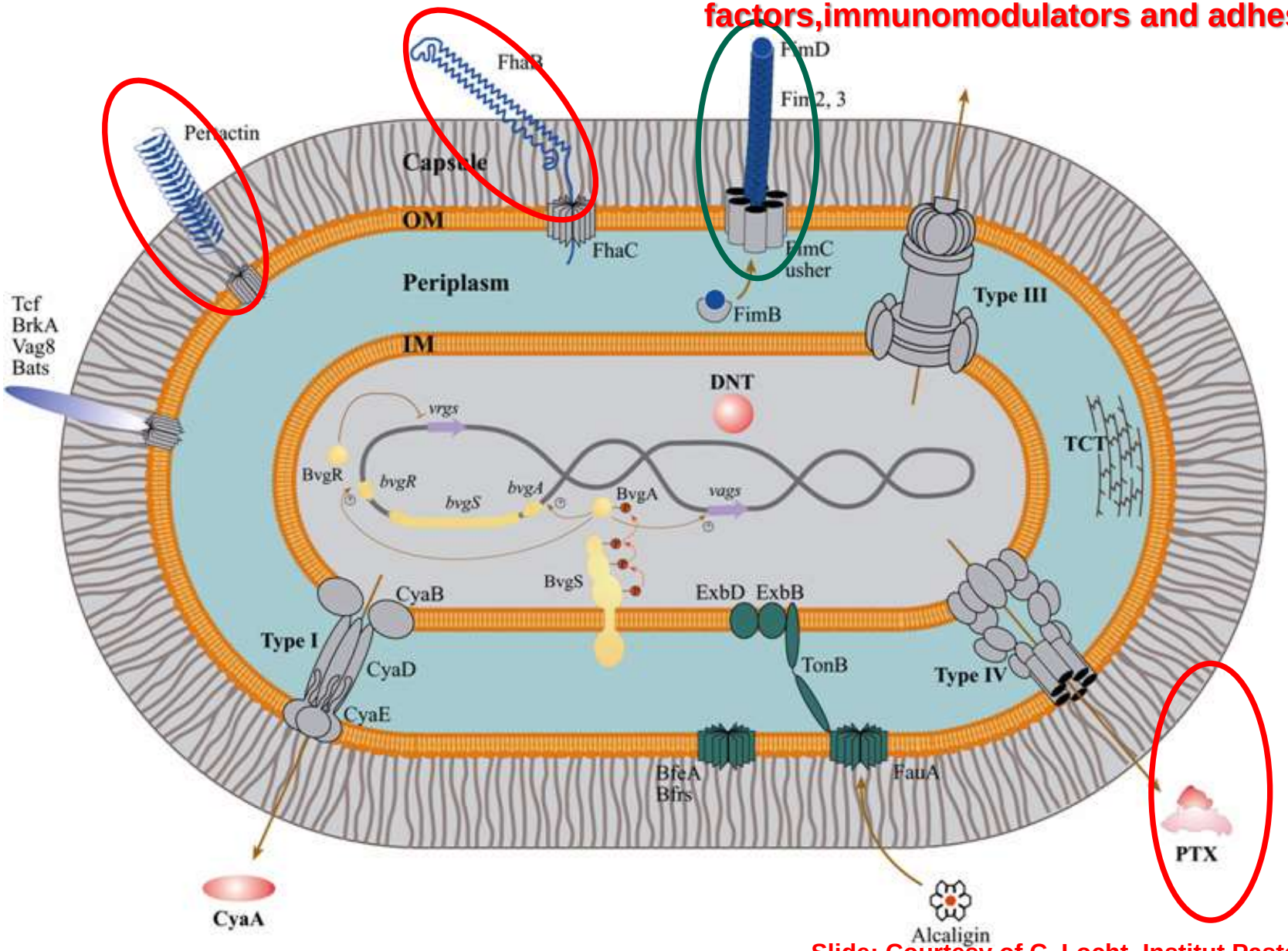
(to the wealthiest countries...)

We`ve got a problem again...

Confirmed clinical pertussis in 1990-2013 in CR (10 million people)
We had 2,518 cases in 2014 (like in 1961…)



B. pertussis is armed with numerous parallel and redundant virulence systems (cytotoxins, complement resistance factors,immunomodulators and adhesins)



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

aP vaccines can be improved, but...

only a next generation of non-reactogenic wP vaccine will be able to stop the spread of the bug in the wealthiest populations

be it as a as booster in older kids and adults – inducing sterilizing immunity
or as priming in a pediatric hexavaccine

ACT relevance for pertussis vaccines

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. :

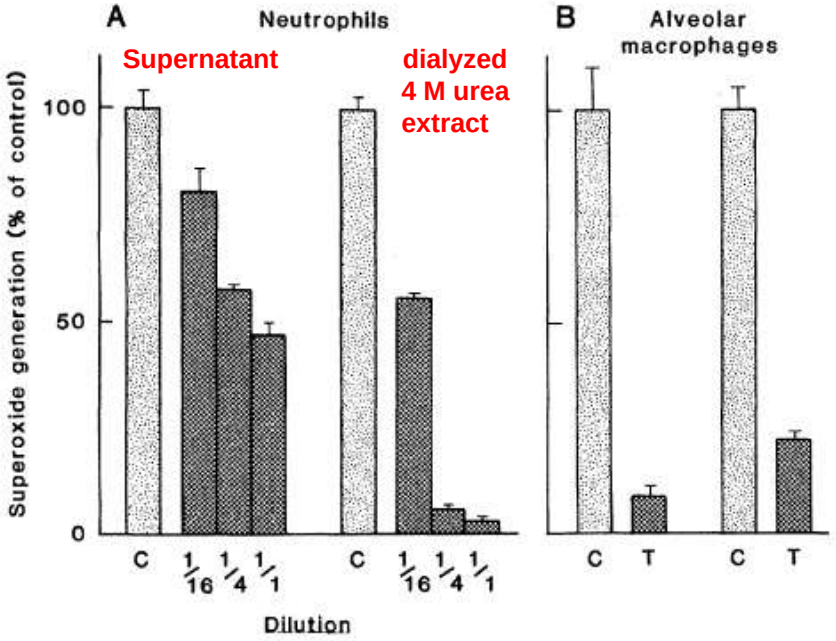


Fig. 1. Superoxide generation by stimulated human phagocytes and inhibition by *Bordetella* products. (A) Human neutrophils, 2×10^6, suspended in 200 μl of Hanks balanced salt solution, were incubated for 10 minutes at 37°C with 200 μl of the indicated dilution of the supernatant of 48-hour cultures 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. :

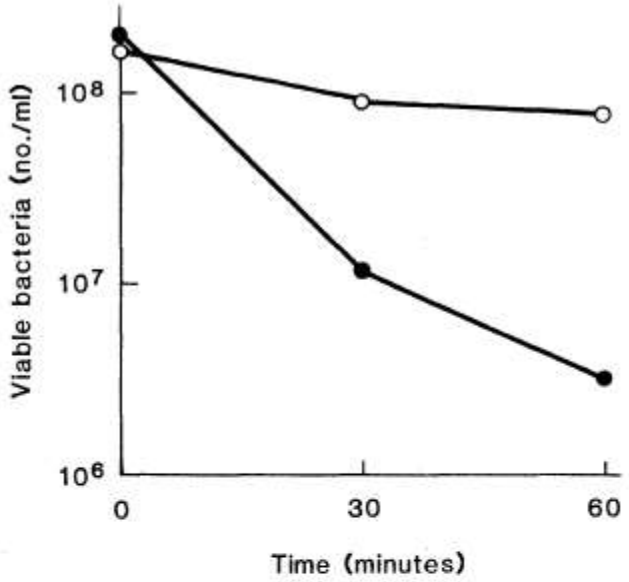


Fig. 2. Neutrophil killing defect induced by *Bordetella* extract. Human neutrophils (2×10^7 per milliliter) suspended in Hanks balanced salt solution were incubated for 5 minutes at 37°C with an equal volume of dialyzed *Bordetella* extract or dialysate control. The killing of *Staphylococcus aureus* 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.

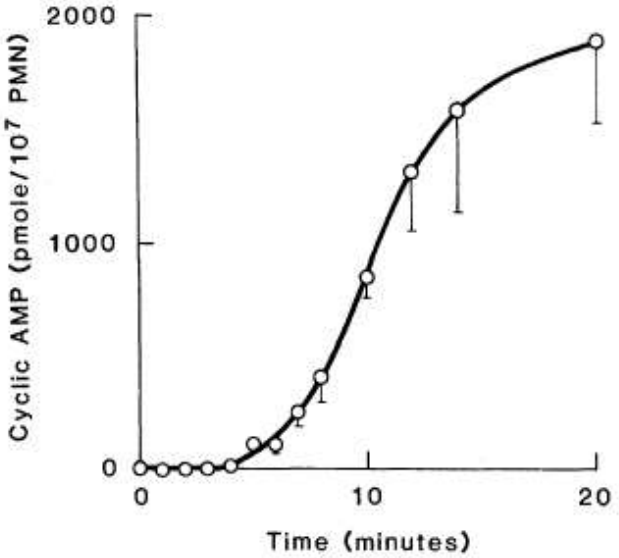


Fig. 3. Accumulation of cyclic AMP in human neutrophils (*PMN*) incubated with dialyzed *Bordetella* extract. Neutrophils, 10^7 per milliliter in Hanks balanced salt solution, were incubated at 37°C with equal volumes of dialyzed *Bordetella* extract (protein content, 520 μg/ml) for the times shown. 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.

Nobody doubts that antibodies against PT save infant`s lifes

= PT needs to be in the aP vaccine

but:

B. parapertussis does not need PT to cause whooping cough

and

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

Goodwin MS, Weiss AA. (1990) *Infect Immun*. 58:3445-7

Khelef N, Sakamoto H, Guiso N. (1992) Microb. Pathog. 12:227-35

The bug really needs ACT for knocking down innate immunity:

- All *Bordetellae* pathogenic to mammals produce adenylate cyclase toxin-hemolysin (Except for certain *B. bronchispetica* lineages)
- ACT sequences are highly conserved in *B. pertussis* isolates
- Strains not producing ACT have not been isolated from patients, so far (in contrast to PT, FHA or pertactin, which all are dispensable)
- ACT is an extremely potent toxin that **knocks-down phagocytes in 30-60 seconds** (PT needs 30 min to get internalized into cells and effects manifest in 8- 12 h
- ACT **instantaneously blocks oxidative burst of neutrophils** at pM conc. in 30 seconds
- ACT **blocks uptake of complement-opsonized particles** at pM conc.

Not suprisingly, hence, ACT is a protective antigen

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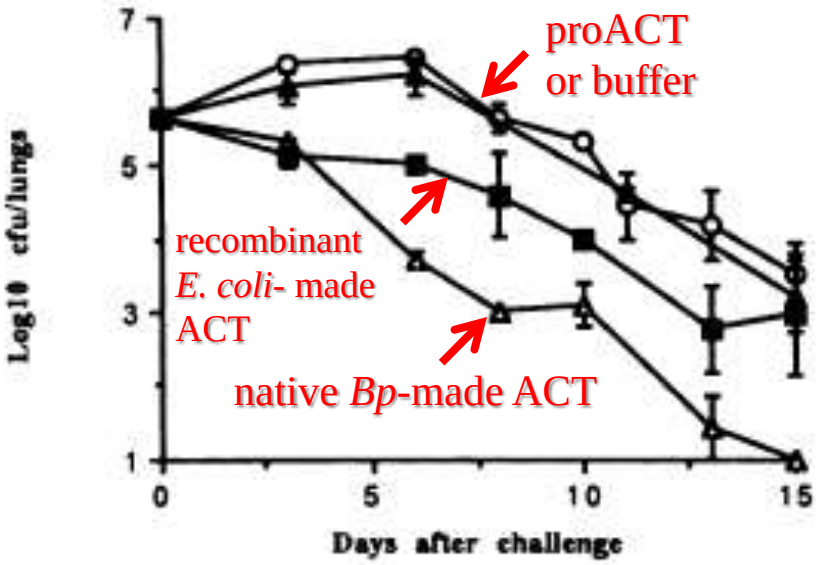
Vol. 61, No. 9

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



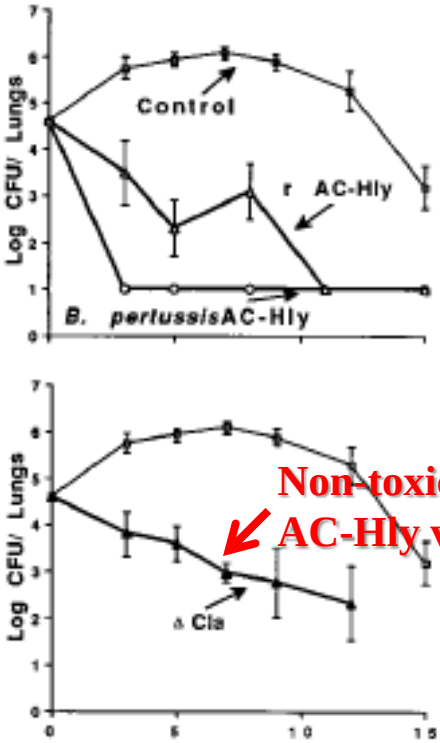
(at that time ACT samples contained LPS)

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 [illegible] 1995



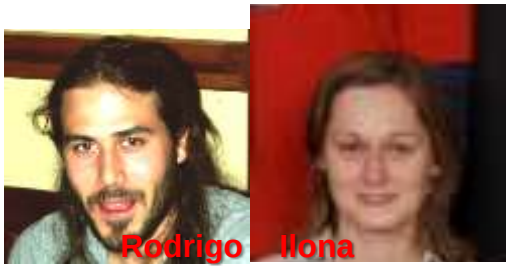
Highly purified CyaA-AC$^-$ protects on its own

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

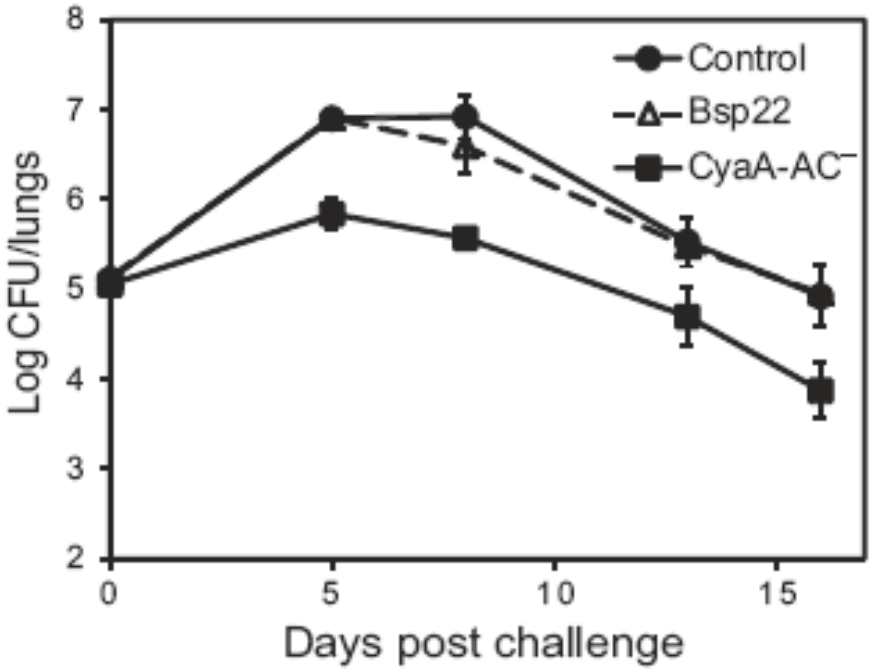
The *Bordetella pertussis* Type III Secretion System Tip Complex Protein Bsp22 Is Not a Protective Antigen and Fails To Elicit Serum Antibody Responses during Infection of Humans and Mice

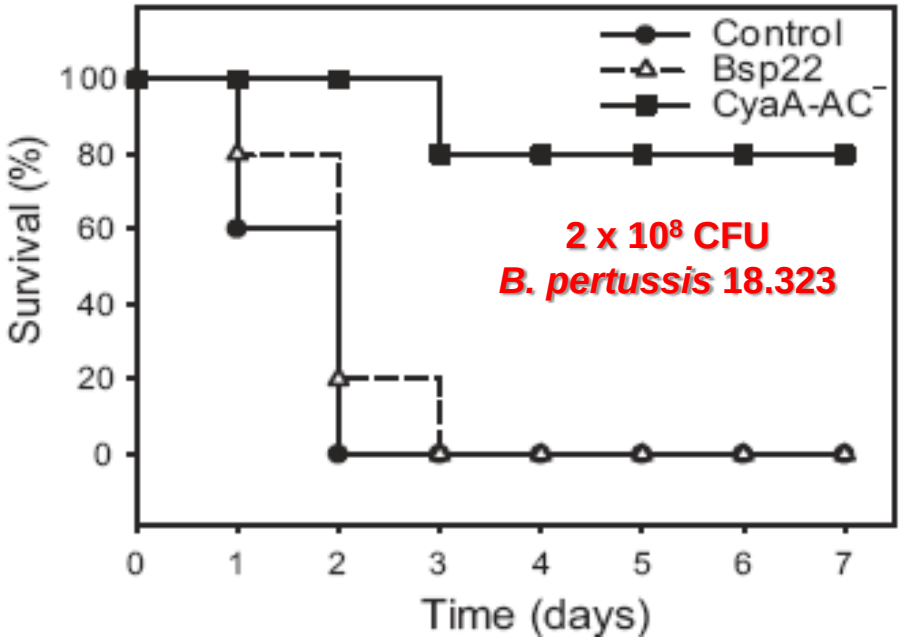
Rodrigo Villarino Romero,[a] Ilona Bibova,[a] Ondrej Cerny,[a] Branislav Vecerek,[a] Tomas Wald,[a] Oldrich Benada,[a] Jana Zavadilova,[b] Radim Osicka,[a] Peter Sebo[a]

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



Poster: Villarino, Bibova *et al.*





Evidence from other labs

- Hormozi K, Parton R, Coote J. **Adjuvant and protective properties of native and recombinant Bordetella pertussis adenylate cyclase toxin** preparations in mice. FEMS Immunol Med Microbiol 1999;23(4): 273-82
- Macdonald-Fyall J, Xing D, Corbel M, et al. **Adjuvanticity of native and detoxified adenylate cyclase toxin** of Bordetella pertussis towards co-administered antigens. Vaccine 2004;22(31-32):4270-81
- Orr B, Douce G, Baillie S, et al. **Adjuvant effects of adenylate cyclase toxin** of Bordetella pertussis after intranasal immunisation of mice. Vaccine 2007;25(1): 64-71
- Cheung GY, Xing D, Prior S, et al. Effect of different forms of adenylate cyclase toxin of Bordetella pertussis on protection afforded by an acellular pertussis vaccine in a murine model. Infect Immun 2006;74(12):6797-805

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[▽]

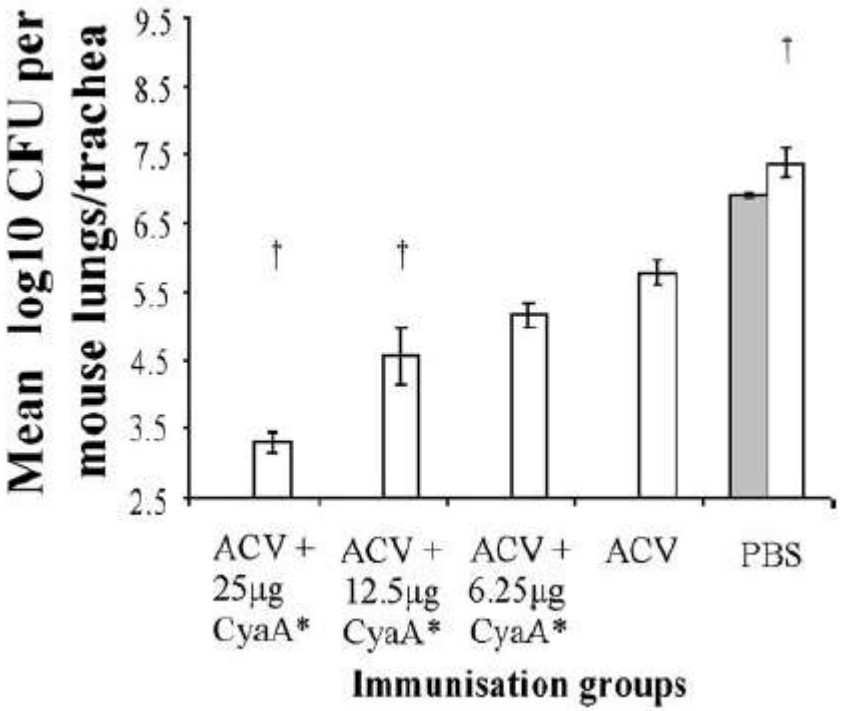
Gordon Y. C. Cheung,[1] Dorothy Xing,[2] Sandra Prior,[2] Michael J. Corbel,[2] Roger Parton,[1] and John G. Coote[1]*

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.



ACT relevance for pertussis vaccines

(Sebo P. *et al.* Expert Rev. Vaccines 13(10), 1215–1227 (2014))

- **ACT is a protective antigen** in the i.n. or aerosol challenge model
- anti-ACT antibodies are common and abundant in convalescent patients

- documented adjuvant effect of the nonenzymatic AC^- toxoid on co-administered B. pertussis antigens

- ACT polarizes T cell responses towards Th1 even when administered with alum!

- AC^- toxoid drives maturation of DC and when mixed with aP vaccine antigens and alum AC toxoid could partly shift the polarization of the immune response from a typical Th2 type, seen with the aP vaccine in mice, to a mixed Th1/Th2 type of response....
  - Cheung GY, Xing D, Prior S, et al. Effect of different forms of adenylate cyclase toxin of Bordetella pertussis on protection afforded by an acellular pertussis vaccine in a murine model. Infect Immun 2006;74(12):6797-805

We shall know in a few years...

GENTICEL
GENTLE T CELL VACCINES

PRESS RELEASE

Paris and Toulouse, February 2nd, 2015

GENTICEL LICENSES VAXICLASE PLATFORM TO SERUM INSTITUTE OF INDIA FOR USE IN PERTUSSIS VACCINES

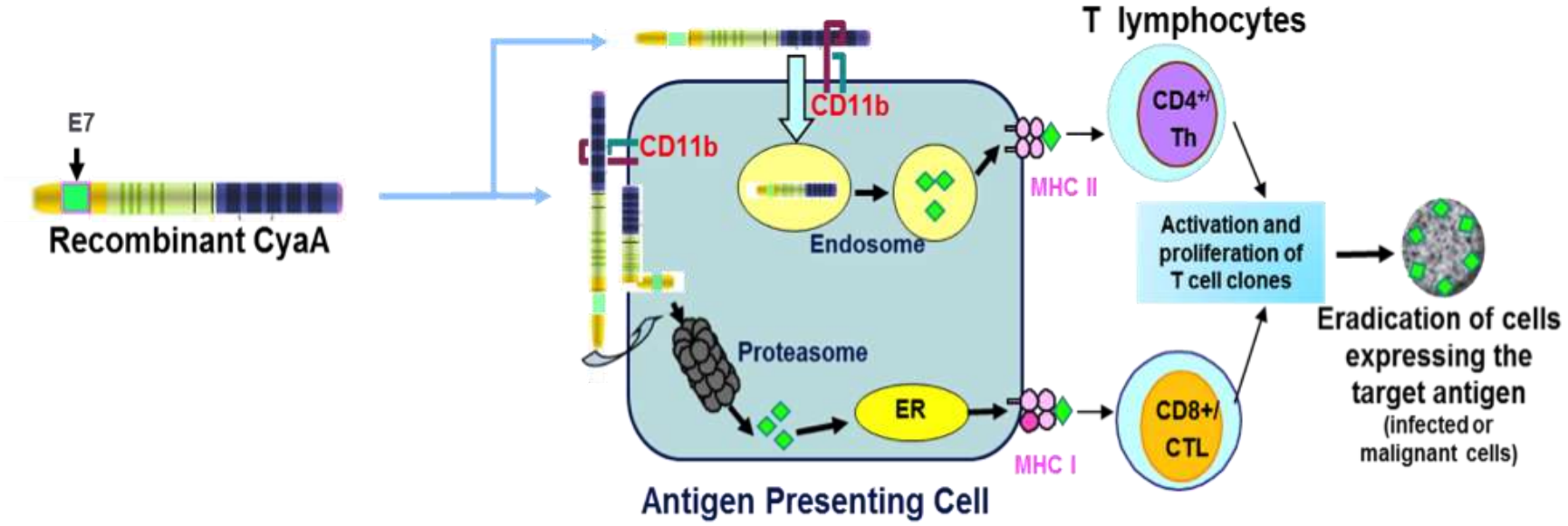
- Serum Institute of India, world's largest producer of vaccines, to evaluate Vaxiclase for use in multivalent vaccines containing pertussis antigens
- Preclinical stage license agreement entitles Genticel to up to $57 million in upfront & milestones payments plus single digit royalties on net sales

GENTICEL (Euronext Paris and Brussels: FR0011790542 - GTCL), a French biotechnology company and leading developer of therapeutic vaccines, today announces that it has licensed its Vaxiclase technology to Serum Institute of India Ltd. (SIIL), for use as a component in acellular multivalent combination vaccines including pertussis antigens.

The license granted by Genticel to SIIL provides the Vaxiclase platform for inclusion in multivalent vaccines that also protect against *Bordetella pertussis*, the causative agent of whooping cough. The license covers all countries of the world except major pharmaceutical markets, in particular the USA, Canada, New Zealand, Australia, Japan, Israel, Turkey and greater Europe.

Vaccines that are built with the CyaA vector are chimeric recombinant proteins consisting of the CyaA protein and the antigen of choice.

1: Sebo P, Fayolle C, d'Andria O, Ladant D, Leclerc C, Ullmann A. (1995) *Infect Immun*. 63(10):3851-7.
2: Fayolle C, Sebo P, Ladant D, Ullmann A, Leclerc C. (1996) J Immunol. 156(12):4697-706.
3. Saron MF, Fayolle C, Sebo P, Ladant D, Ullmann A, Leclerc C. Proc Natl Acad Sci U S A. 1997;94(7):3314-9.



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

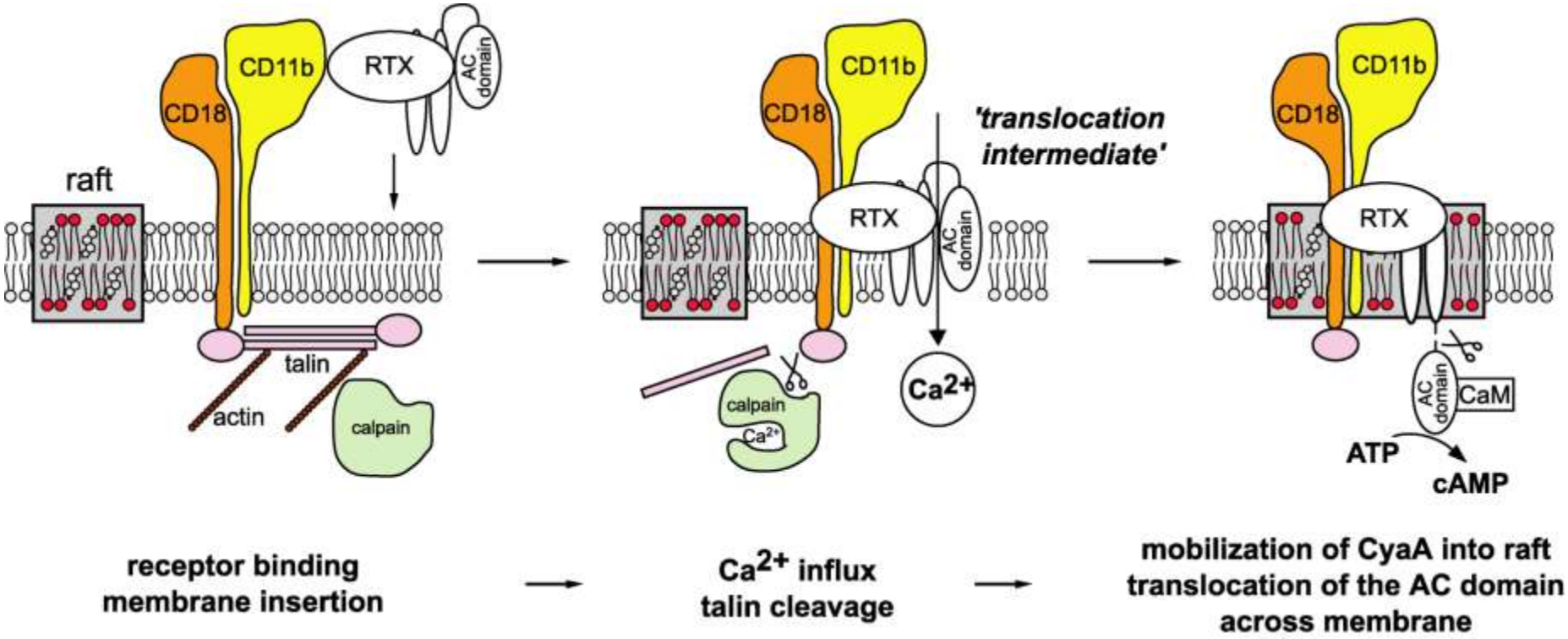
Using a cGMP batch of the adenylate cyclase (CyaA-AC$^-$) toxoid for delivery of HPV E7 antigen as immunotherapeutic vaccine

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

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

IPO on April 4, 2014 at Euronext Paris and Brussels - 34 millions Euro

*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.

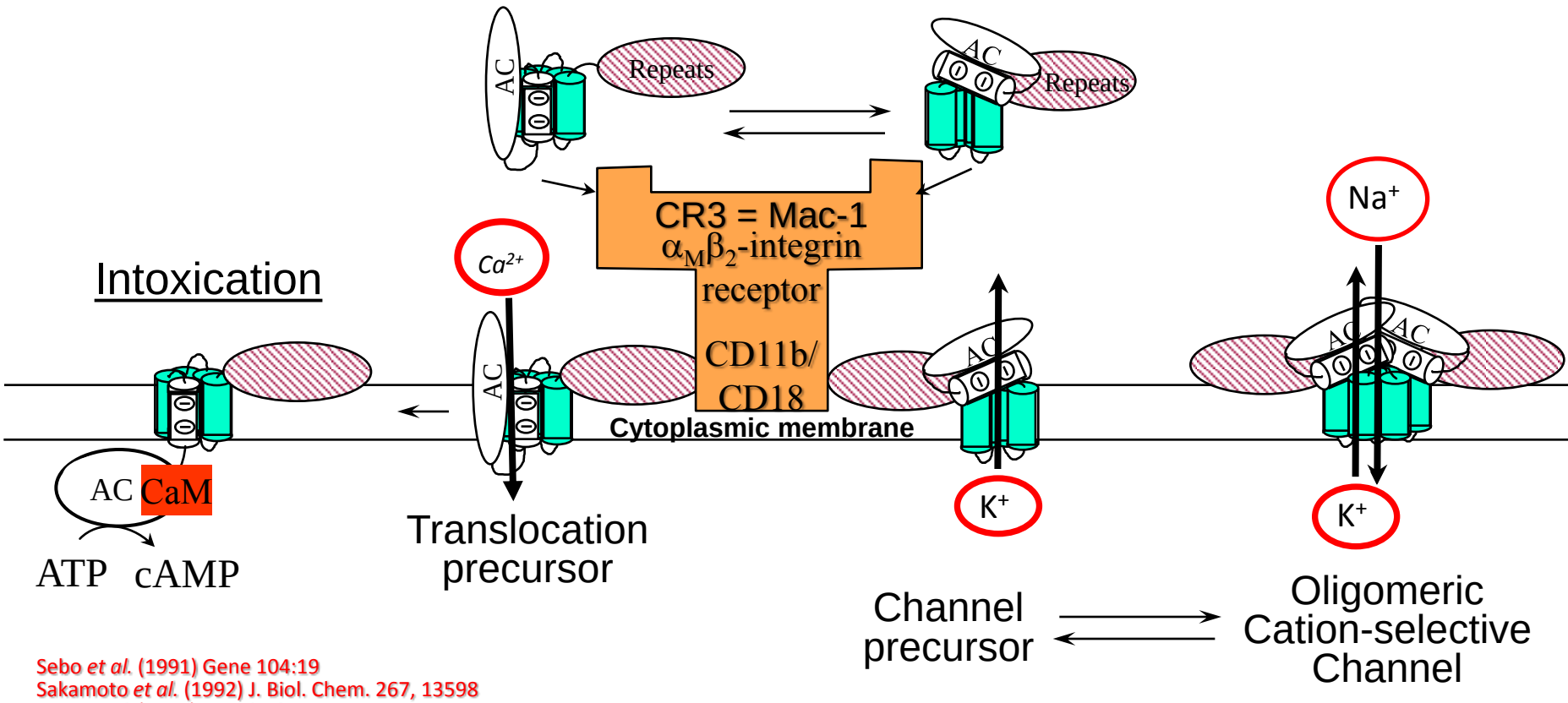
CyaA-AC$^-$ higher concentrations induces maturation of DC through LPS-independent TLR4 and TRIF signaling..

Antigen Targeting to CD11b$^+$ Dendritic Cells in Association with TLR4/TRIF Signaling Promotes Strong CD8$^+$ T Cell Responses

- CyaA binds CD11b+ dendritic cells (DCs) and induces their maturation...
- DCs sense CyaA through the TLR4/Toll/IL-1R domain–containing adapter-inducing IFN-β pathway, independent of the presence of LPS
- leading to the induction of strong immune responses.

Dadaglio *et al.* (2014) J. Immunol. 193: 1787–1798.

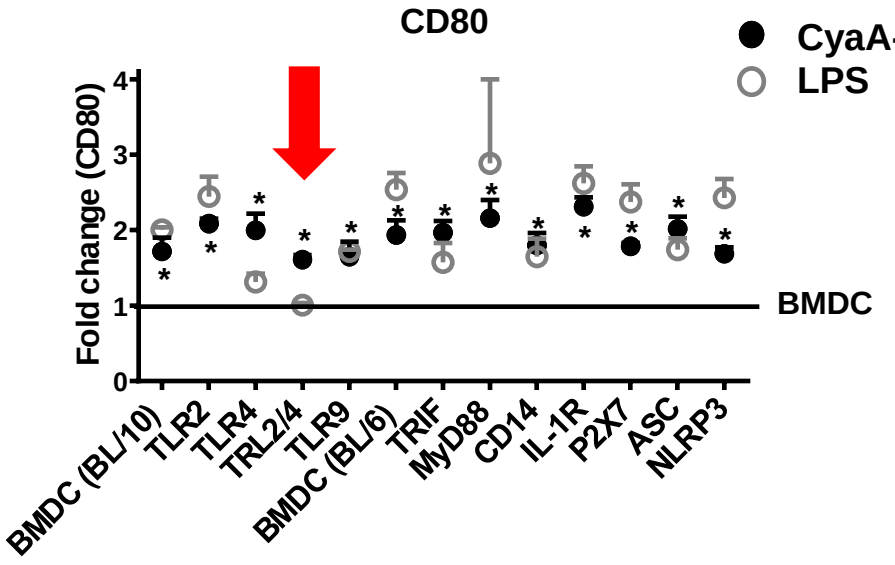
The three cytotoxic activities of ACT adenylate cyclase toxin & pore-forming hemolysin/Cytolysin

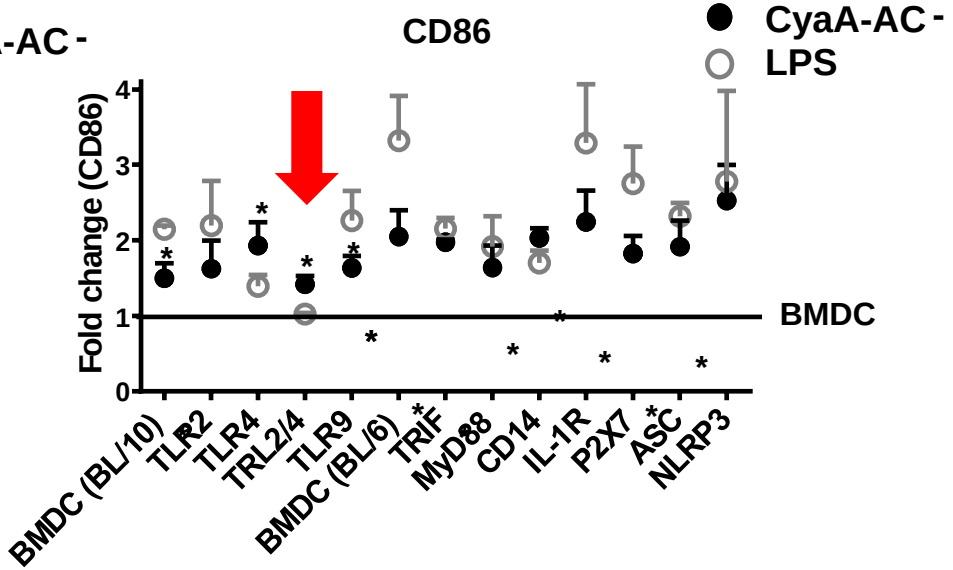


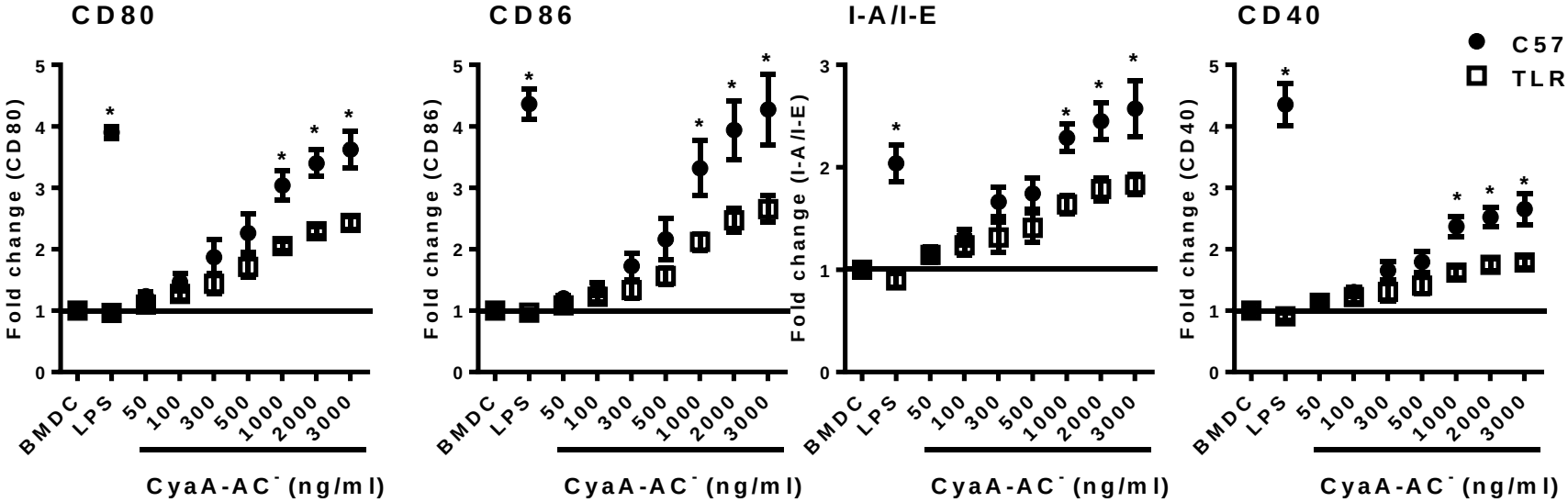
Sebo *et al.* (1991) Gene 104:19
Sakamoto *et al.* (1992) J. Biol. Chem. 267, 13598
Benz *et al.* (1994) J. Biol. Chem. 269, 27231
Hackett *et al.* (1995) J. Biol. Chem. 270, 20250
Gray *et al.* (1998) J. Biol. Chem. 273, 18260
Osickova *et al.* (1999) J. Biol. Chem. 274, 37644
Basler *et al.* (2007)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

However, already cell permeabilization by the Pore-forming activity by low amounts of CyaA-AC$^-$ induces maturation of DC

(Svedova et al. 2015 Immun Cell Biol, in press)

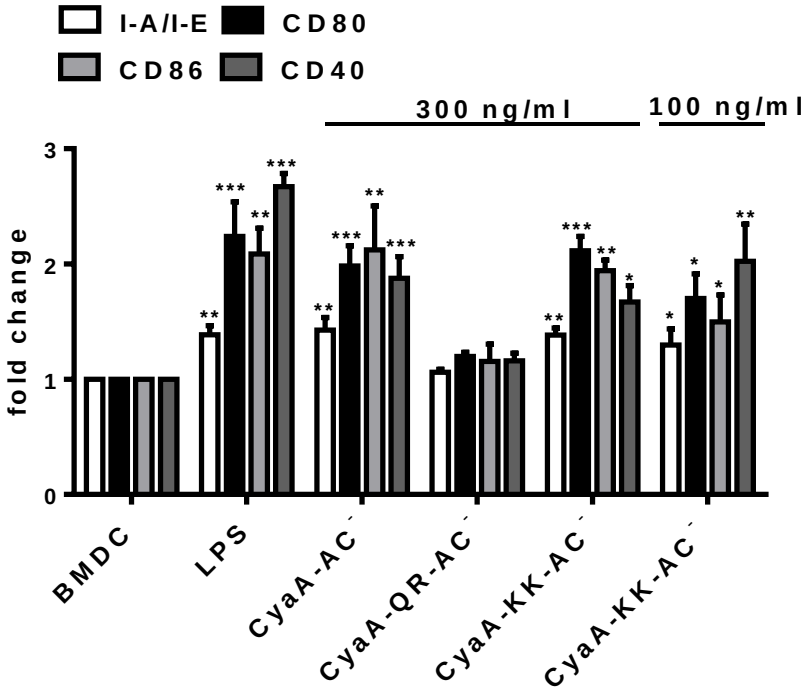


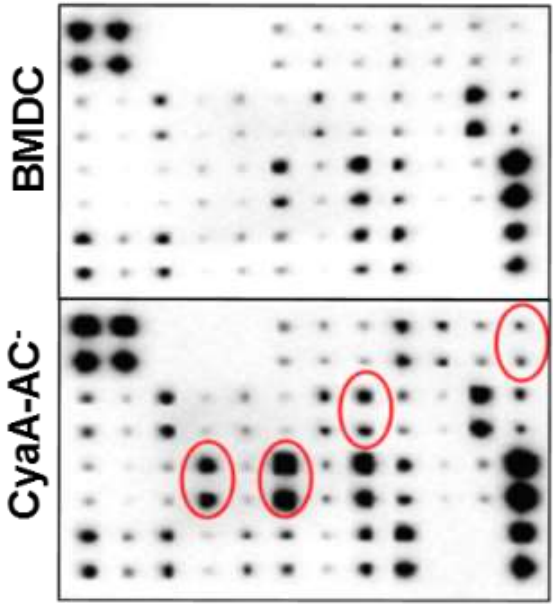


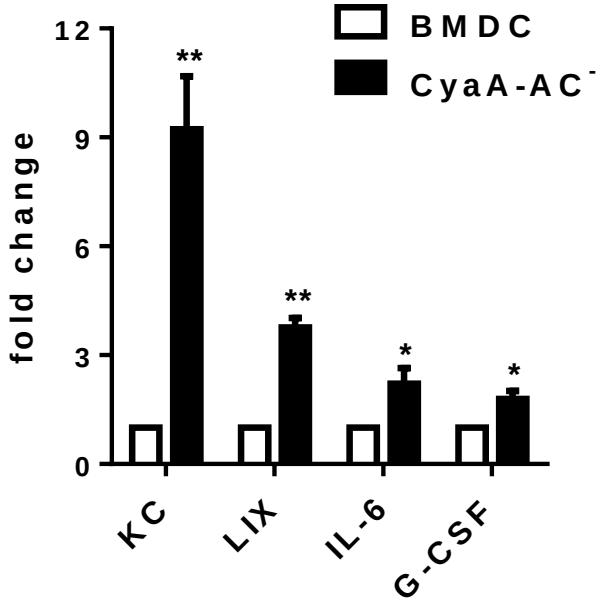


Toxoid activates DC in function of Pore-forming activity (K^+ efflux)

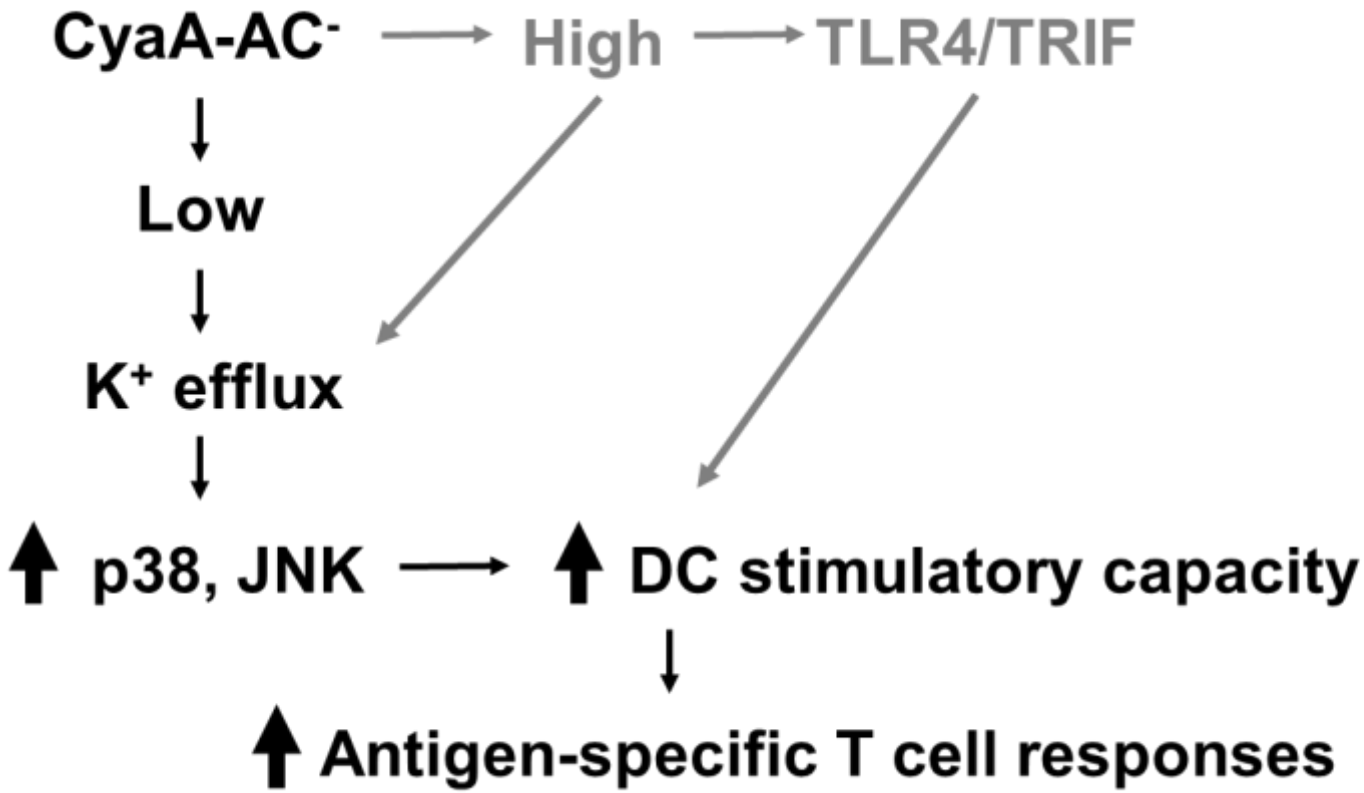
CyaA mutants	Ca^{2+} influx	Specific cell-permeabilizing activity (K^+ efflux)
CyaA-AC$^-$	+++	++
CyaA-E570Q-K860R-AC$^-$	+++	-/+
CyaA-E509K-E516K-AC$^-$	-/+	++++







The CyaA-AC$^-$ Toxoid primes activation of DC by cell-permeabilization, causing K^+ efflux and p38 MAPK activation. At higher toxoid concentrations, CyaA-mediated clustering of CD11b/CD18 with TLR4 and TRIF signaling occurs

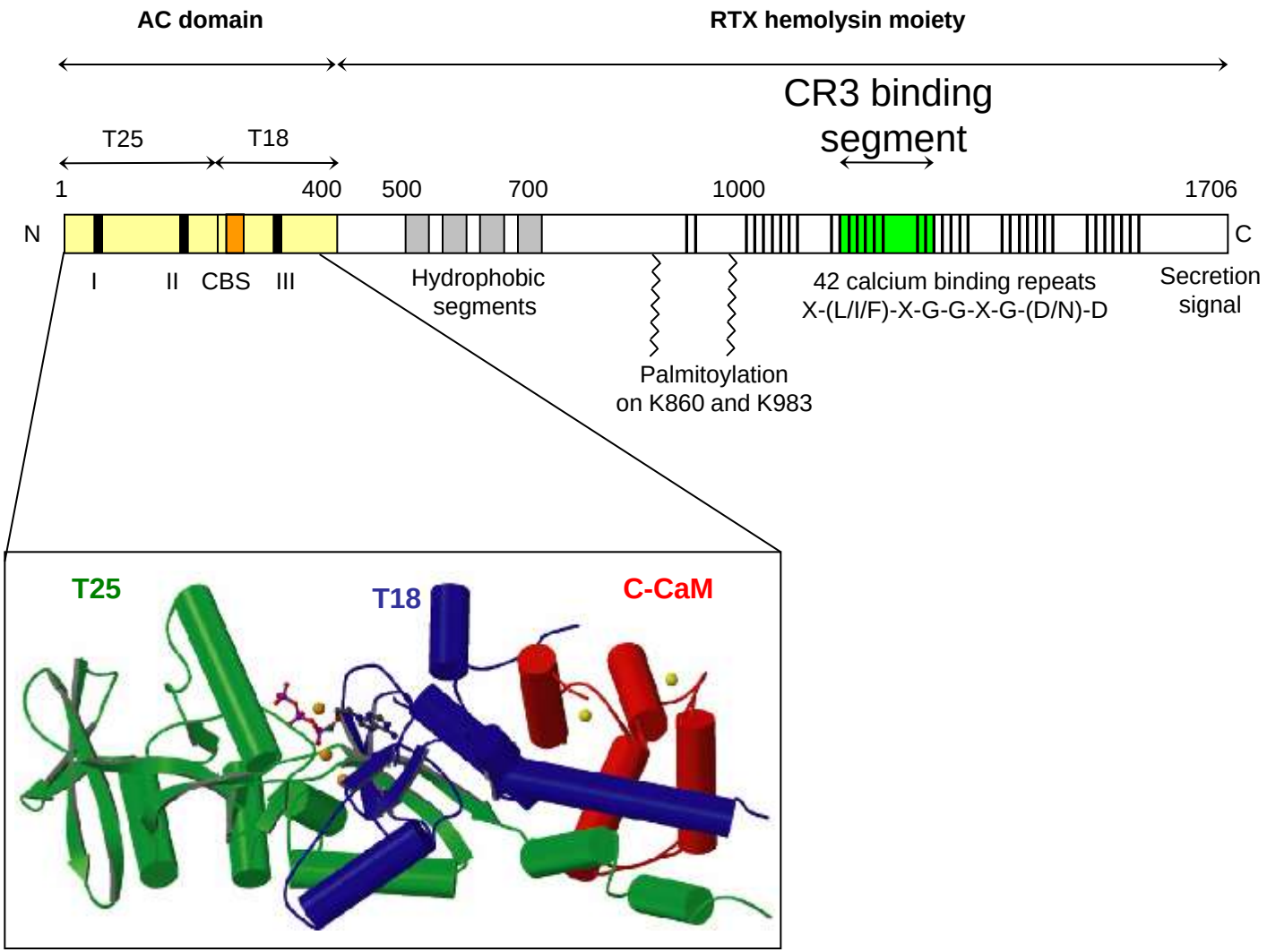


Svedova et al., (2015) Immunology and Cell Biology in press

So, why is ACT so much relevant for pertussis vaccines?

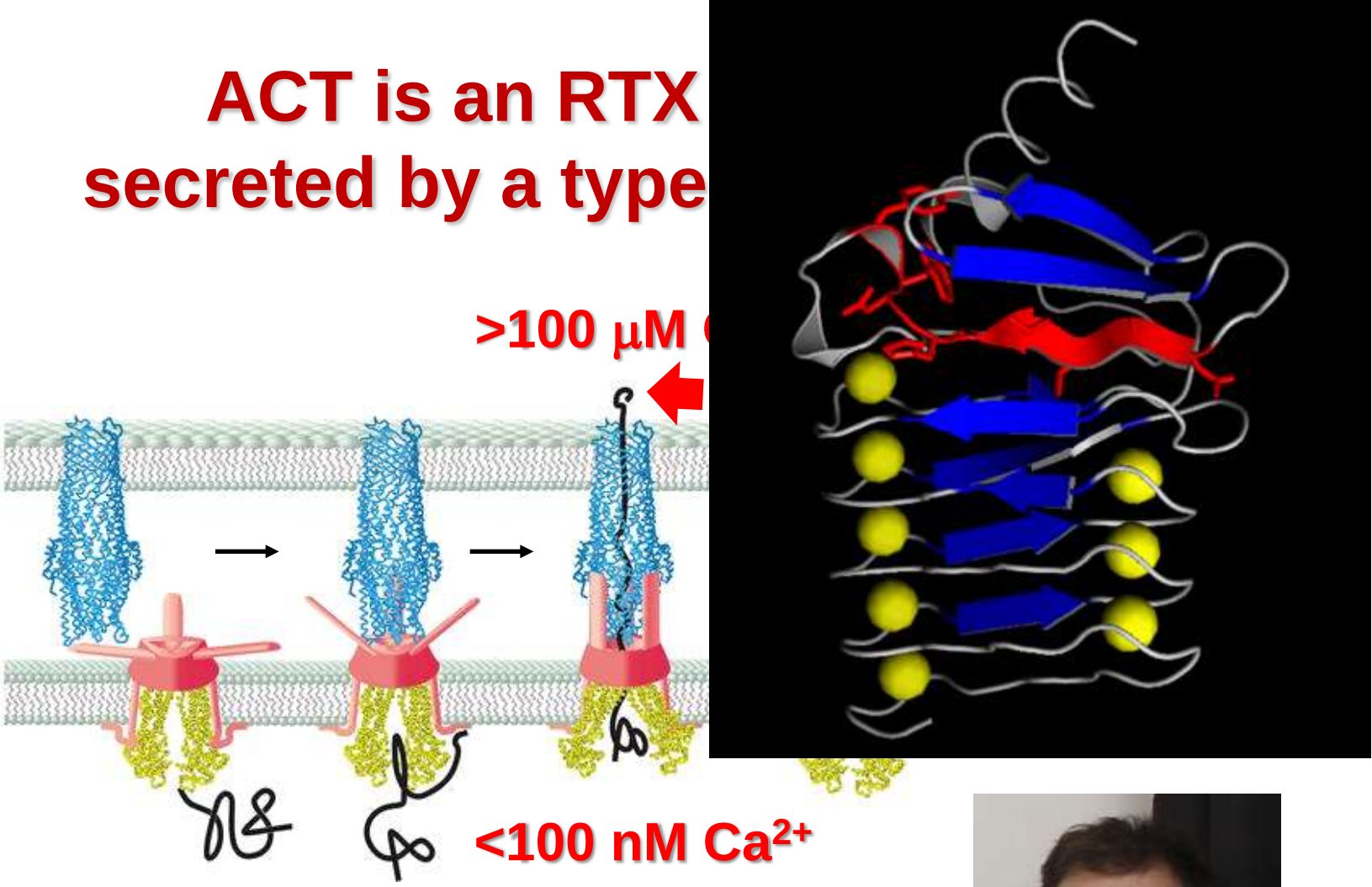
What does it do and how does it work?

Adenylate cyclase toxin - cytolysin



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

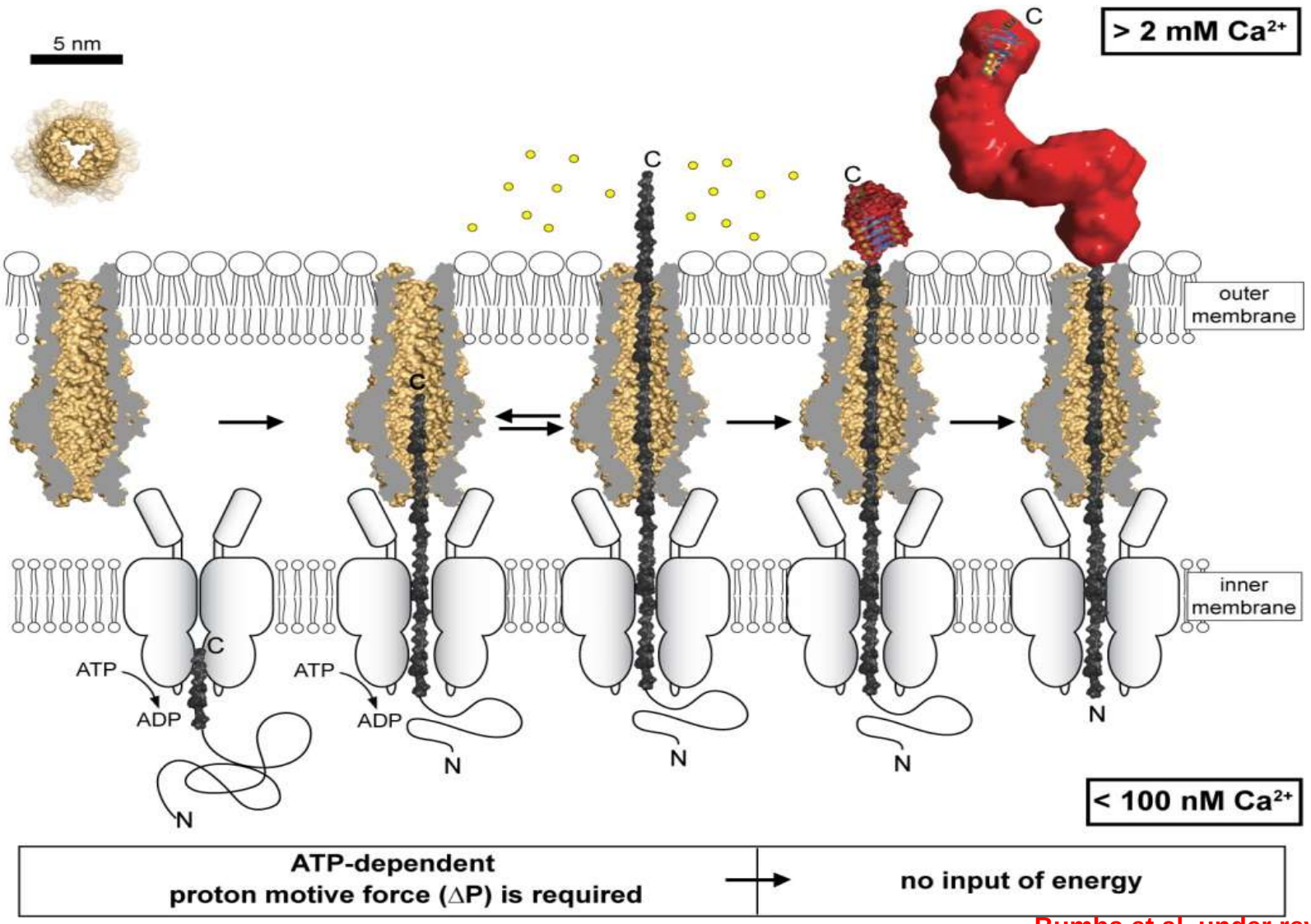
ACT is an RTX
secreted by a type



Need to unfold and refold on the way to target…

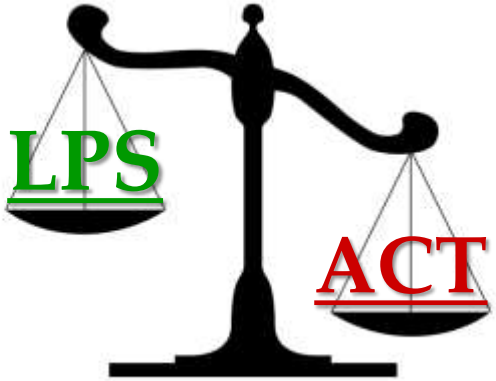
Láďa

Calcium-driven formation of an intramolecular Brownian ratchet directs movement of large RTX proteins through type I secretion system conduits



Bumba et al. under revision

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



↓ ciliary beating

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

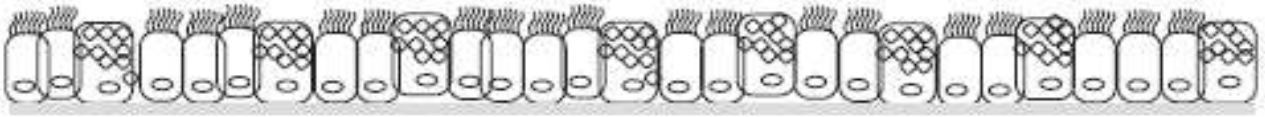
signal transduction events:
NF-κB, ↓ MAPK – p38, ERK, JNK

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

mucin: MUC2, MUC5AC ↑

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

AEC



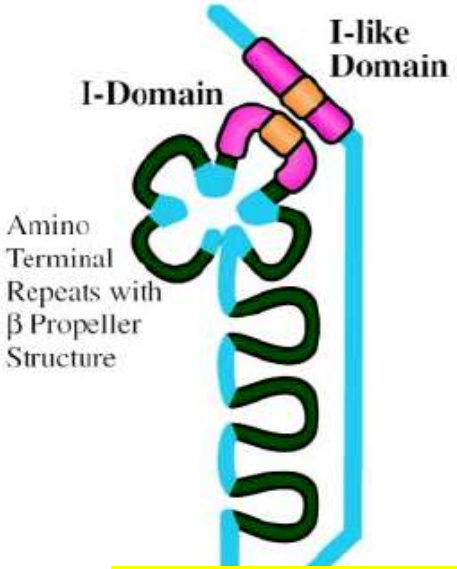
cAMP

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

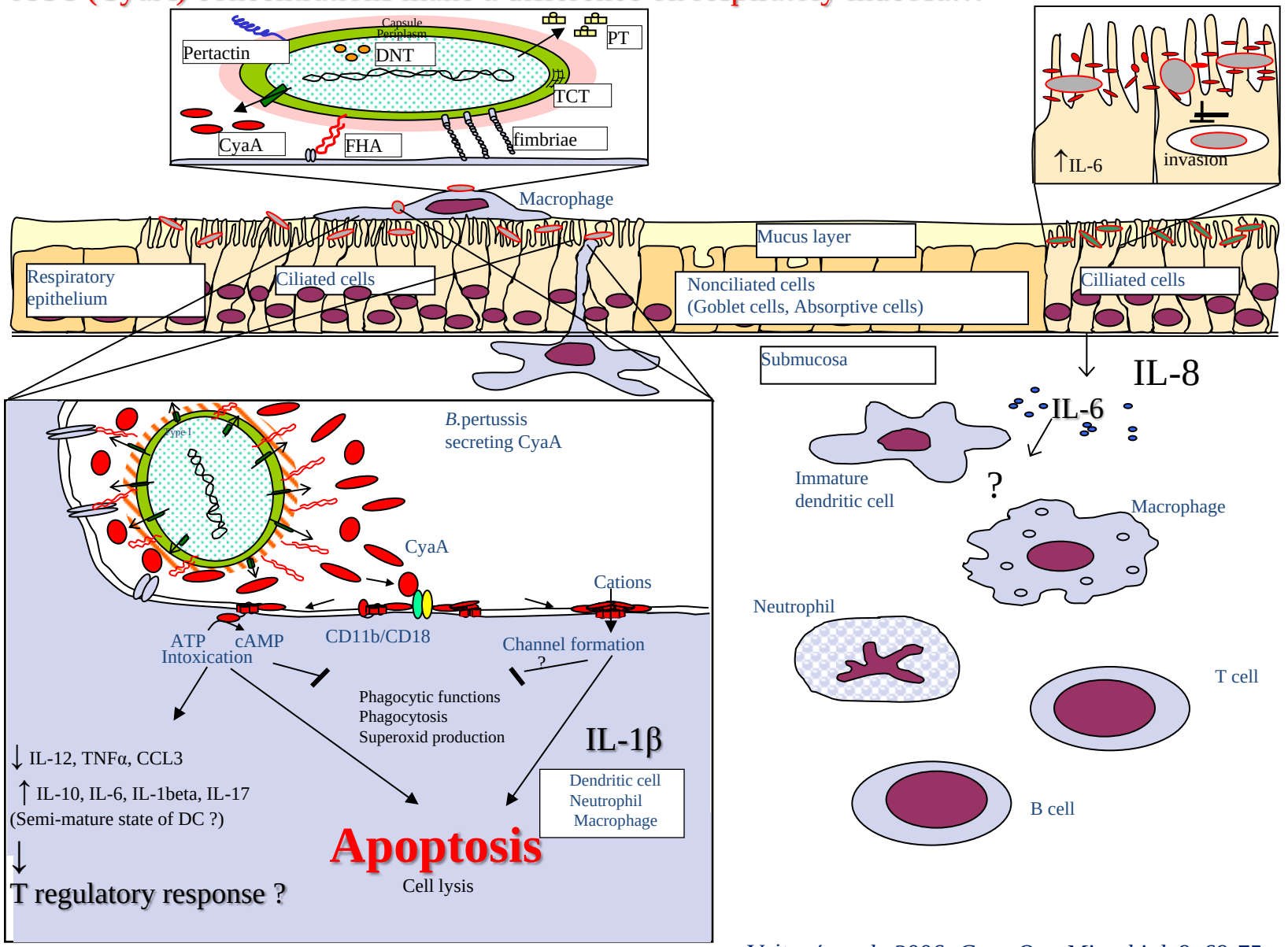
ACT first recognizes N-linked glycans of CD11b/CD18

Morova et al. (2008) PNAS 105, 5355

Guermonprez et al. 2001, J Exp. Med.

the Yin: ACT as a SWIFT SABOTEUR

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



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

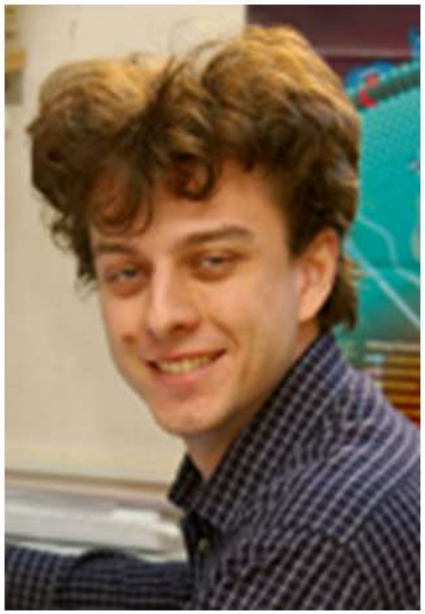
Vojtová *et al.*, 2006, *Curr. Op.. Microbiol.* 9, 69-75

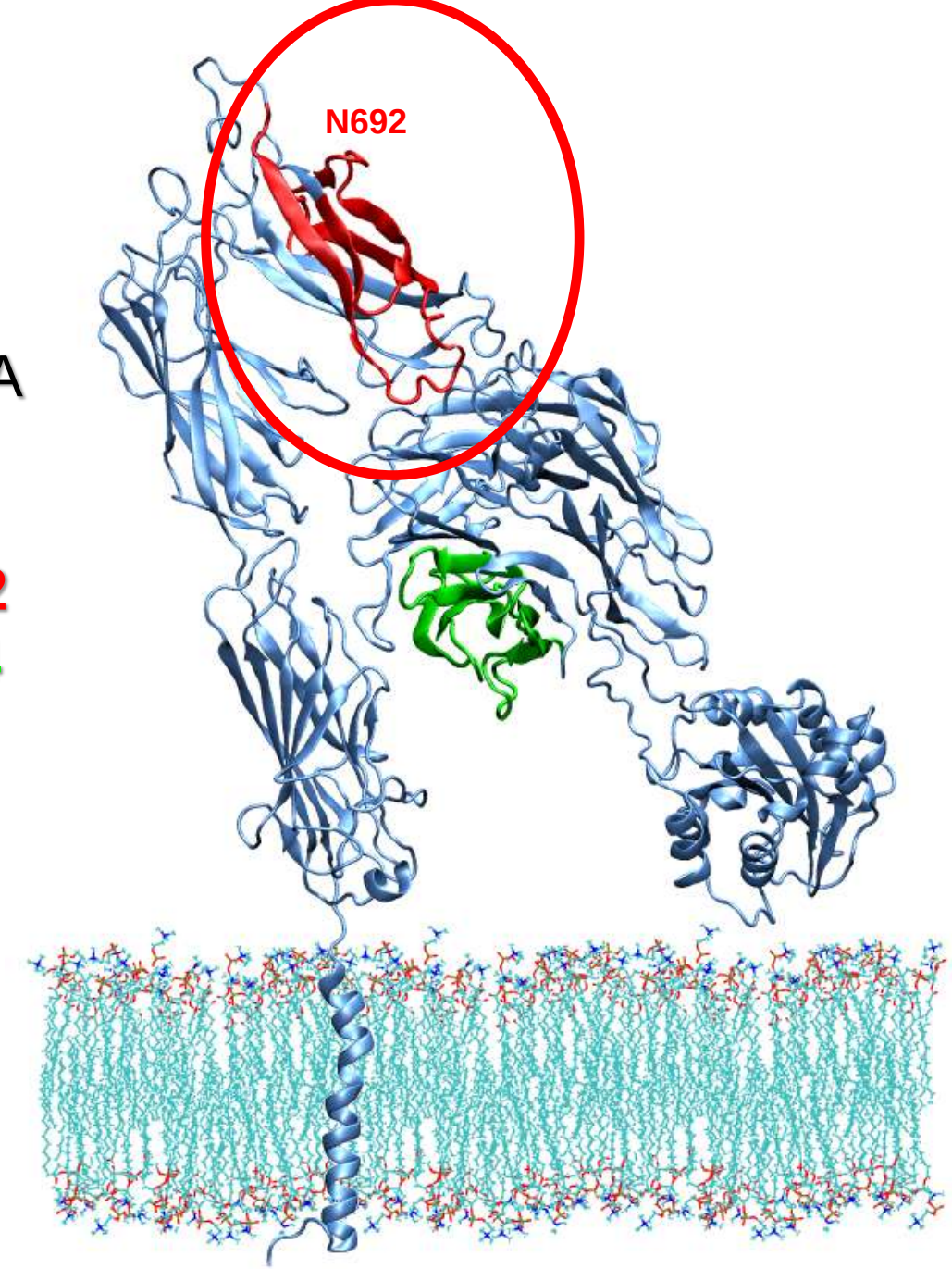
Signaling disarming phagocytes

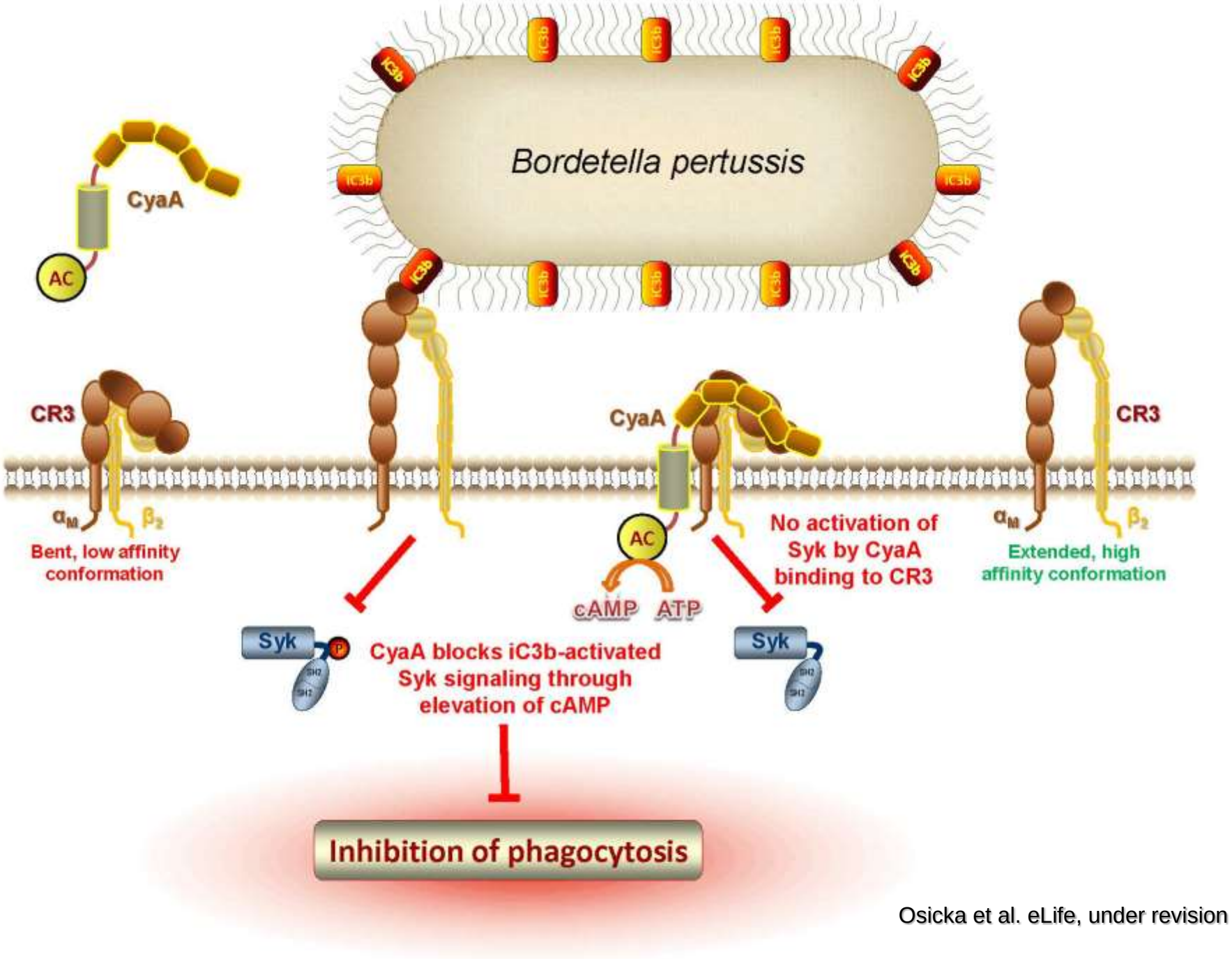
Proteinaceous segments Specifically involved in CyaA binding:

CD11b – residues 614-682

CD11b - residues 342-424

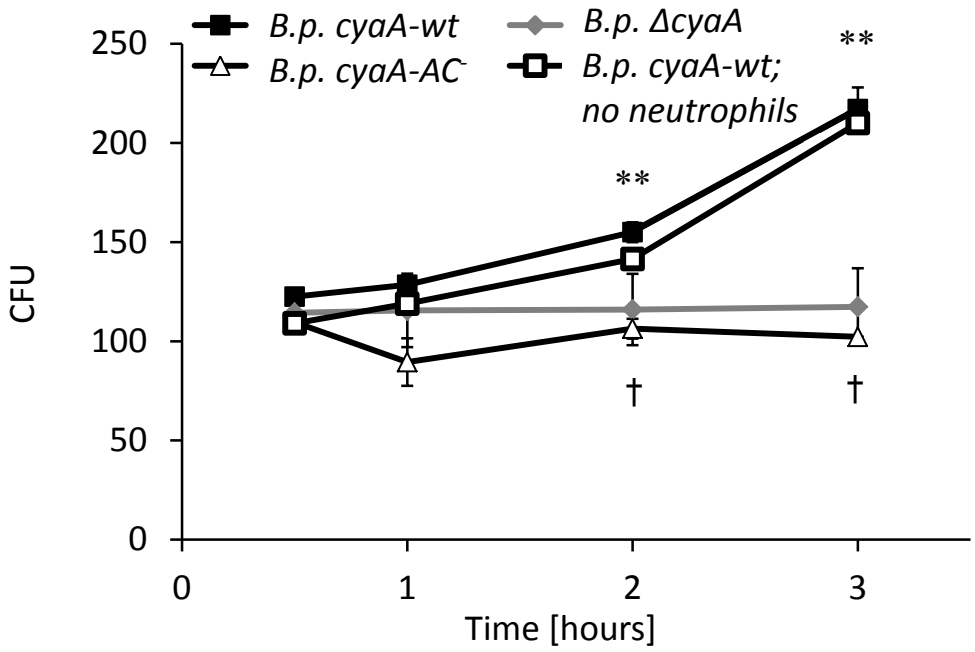






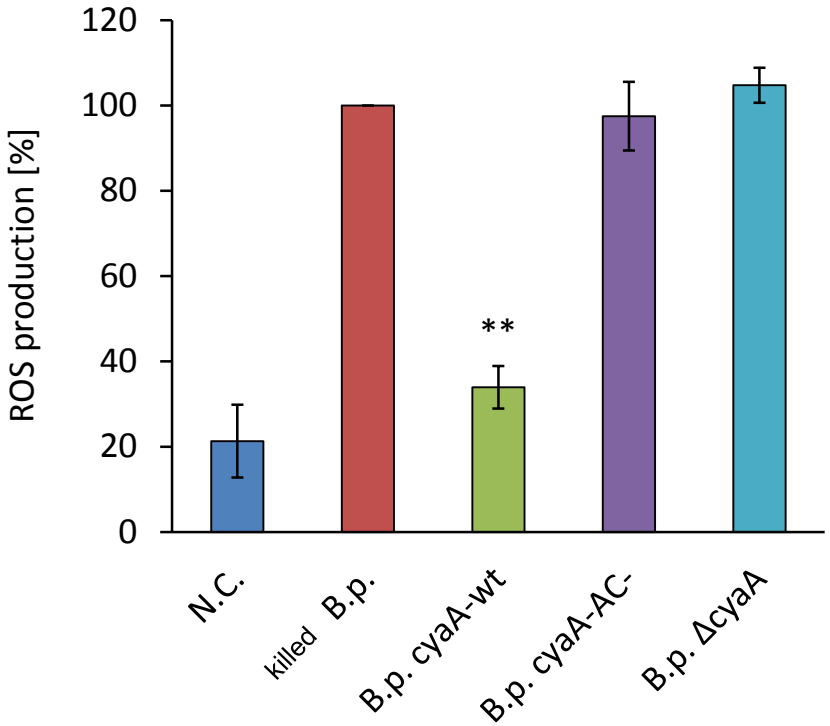
Osicka et al. eLife, under revision

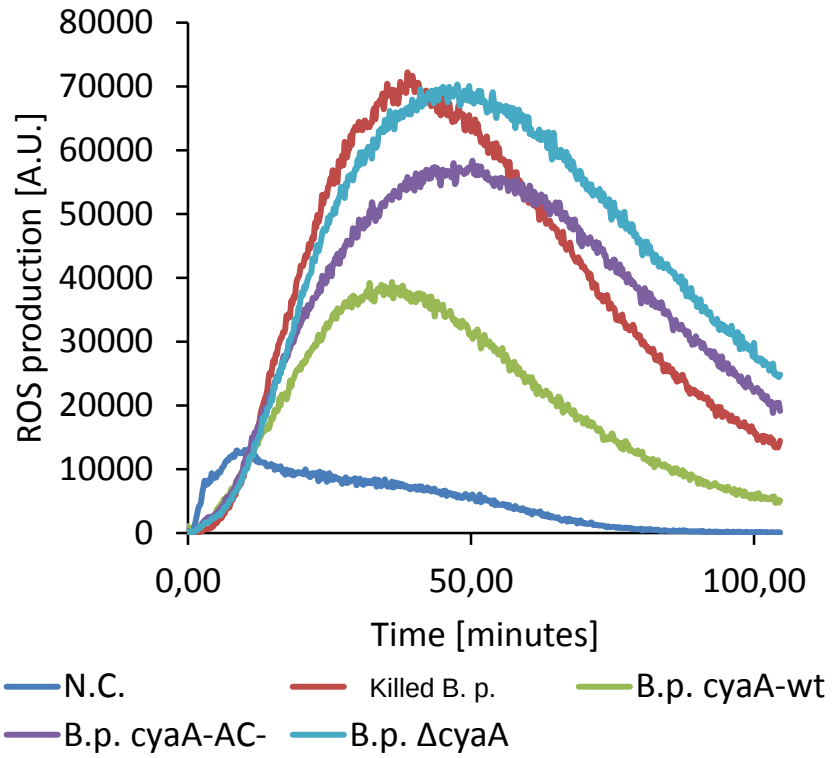
cAMP-elevating AC activity of ACT (CyaA) enables B. pertussis growth in the presence of neutrophils



Cerny et al., unpublished

AC activity of *B. pertussis* ACT blocks ROS production by neutrophils and NET formation





Cerny et al. unpublished

Eby JC, Gray MC, Hewlett EL (2014). Infect Immun. 82:5256-69.

- **cAMP-mediated suppression of neutrophil extracellular trap formation and apoptosis by the *B. pertussis* adenylate cyclase toxin.**
- **Convalescent-phase antisera from patients recovering from pertussis block ACT-mediated inhibition of the oxidative burst and NET formation**

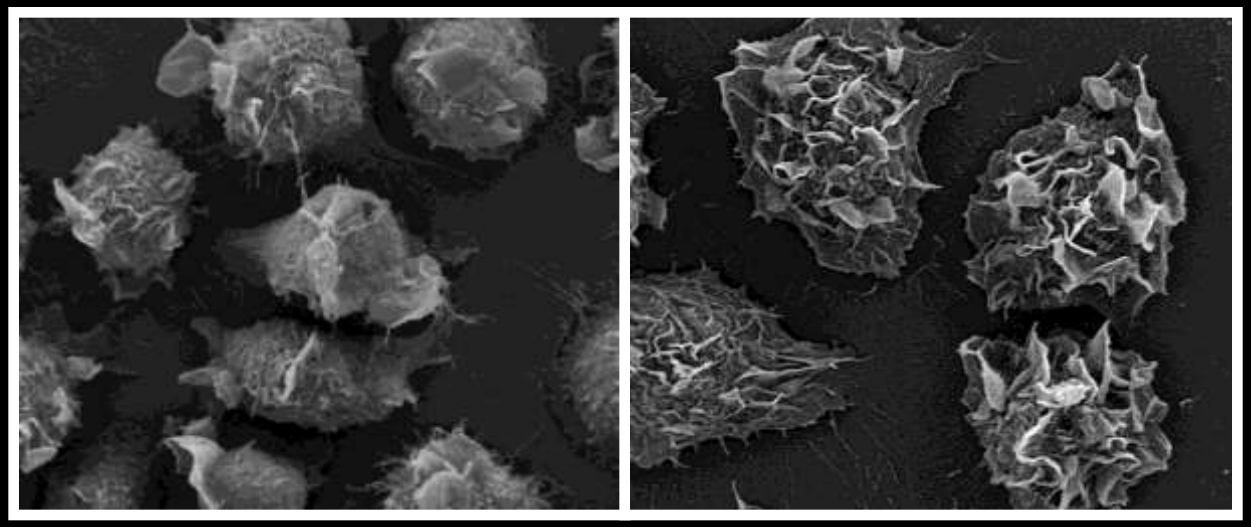
CyaA-induced morphological rearrangements

Mouse macrophage-like cell line J774 A.1:

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

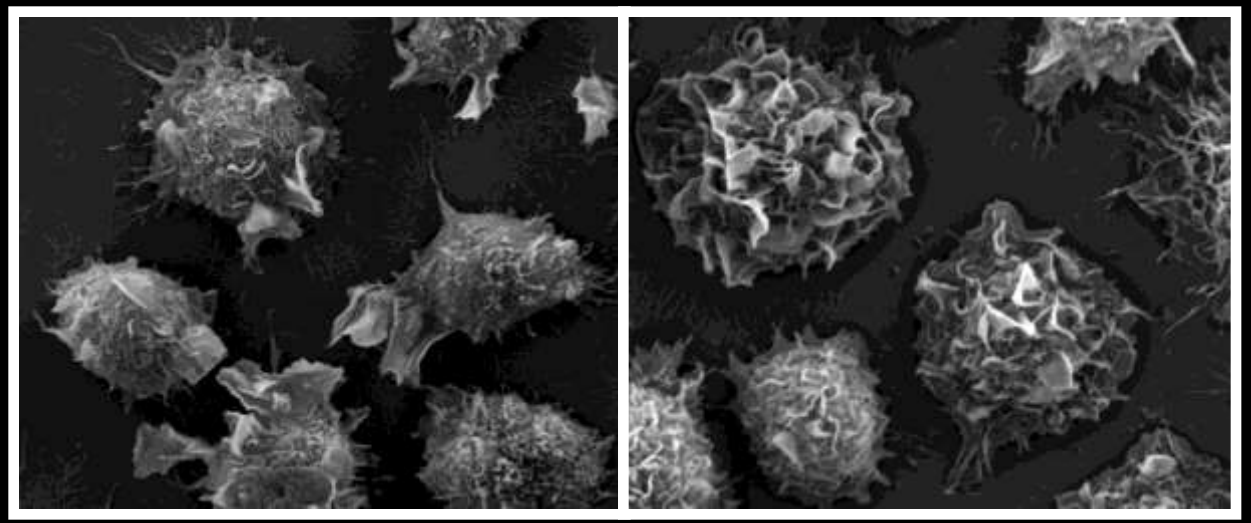
Buffer, 5 min

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

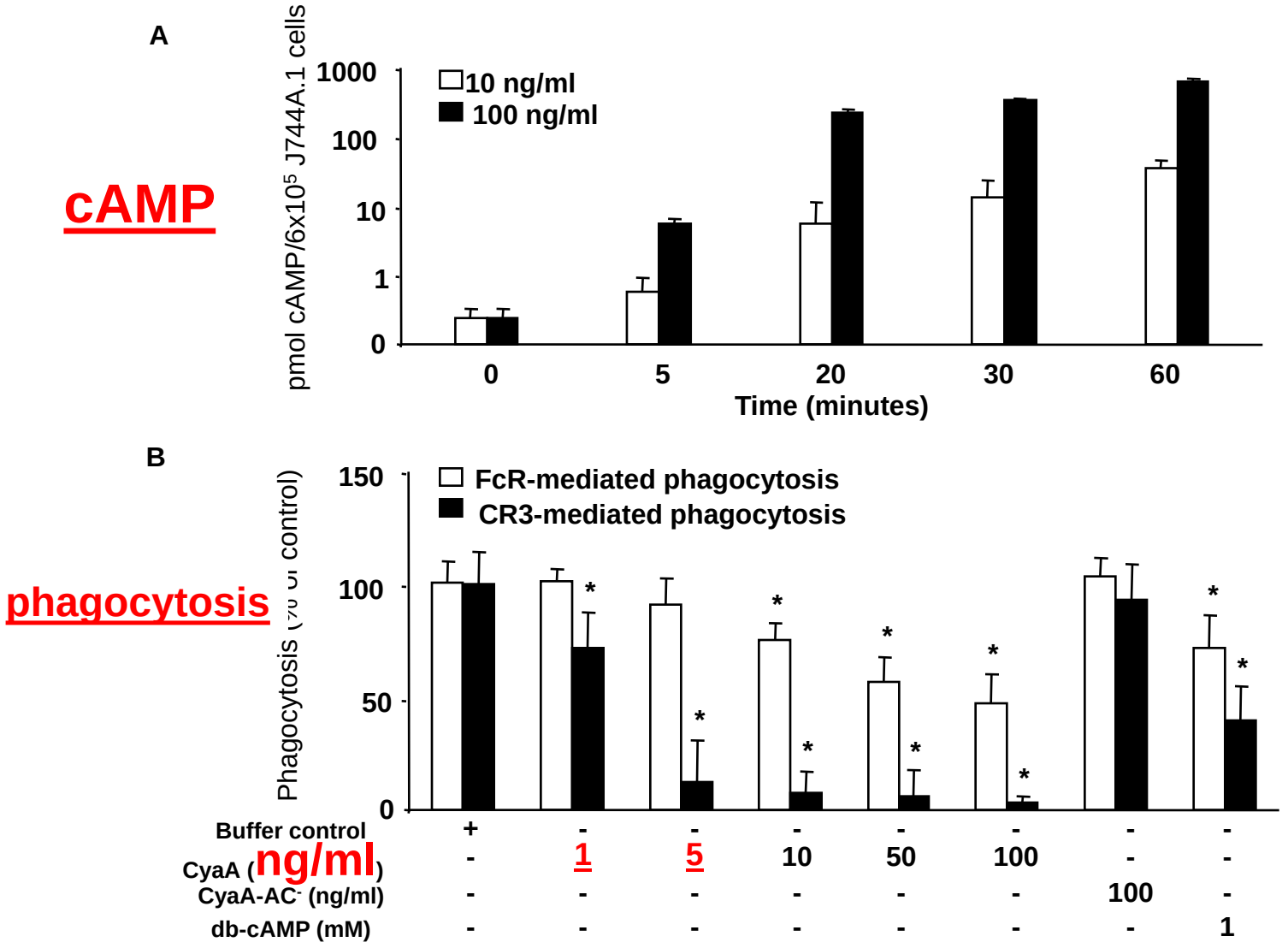


CyaA-AC$^-$, 10 ng/ml, 5 min

db-cAMP, 2mM, 10 min

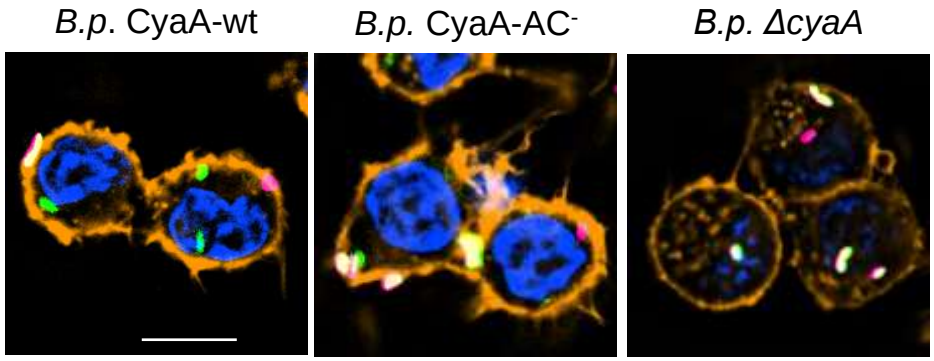


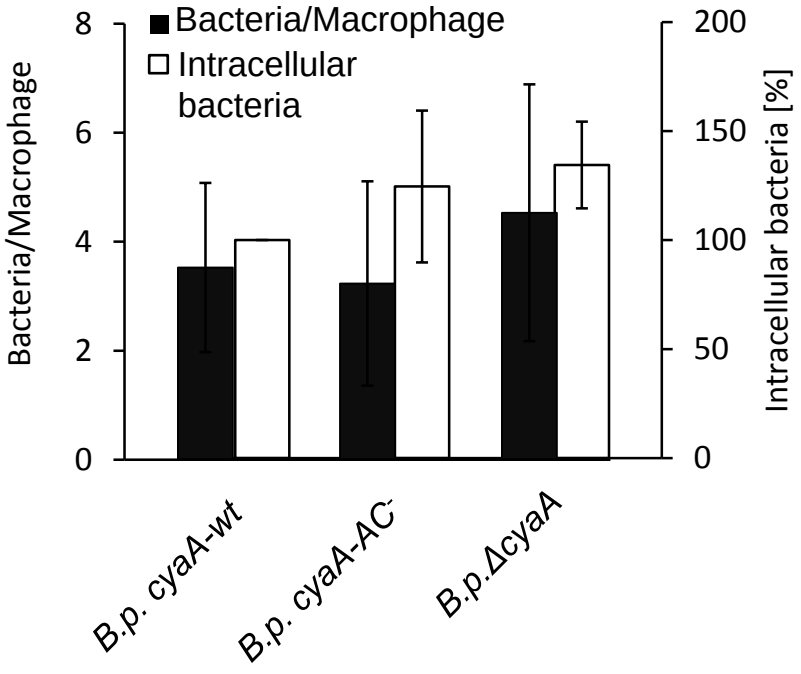
ACT at low doses ablates complement-mediated opsonophagocytosis (through RhoA inactivation)

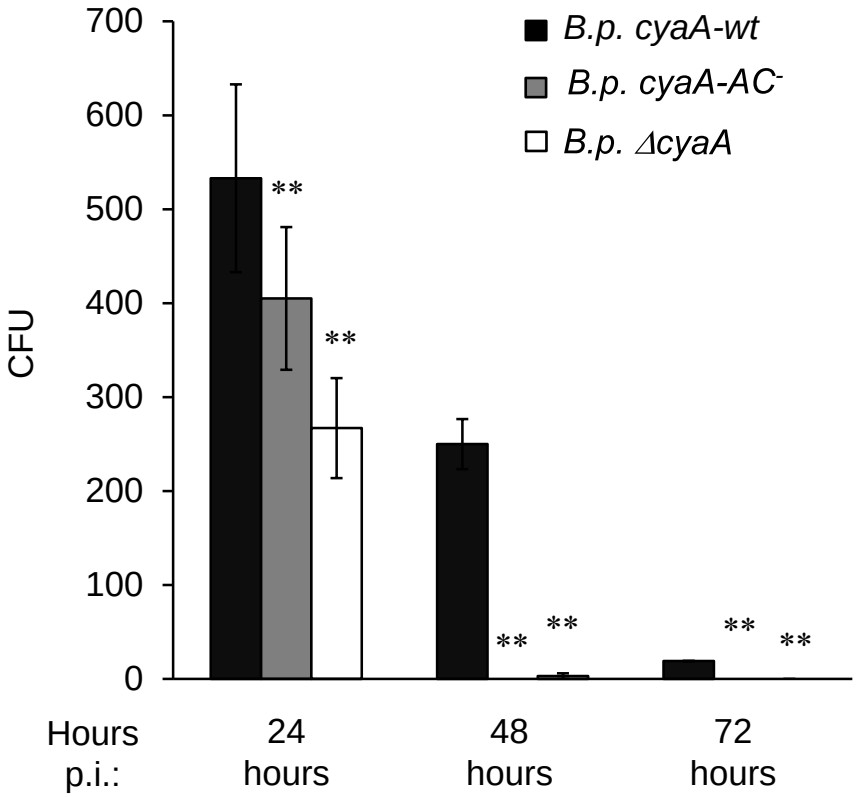


Kamanova et al. (2008) *J. Immunol.* 181, 5587-97

Non-opsonized *B. pertussis* can persist within macrophages due to cAMP elevating activity of ACT

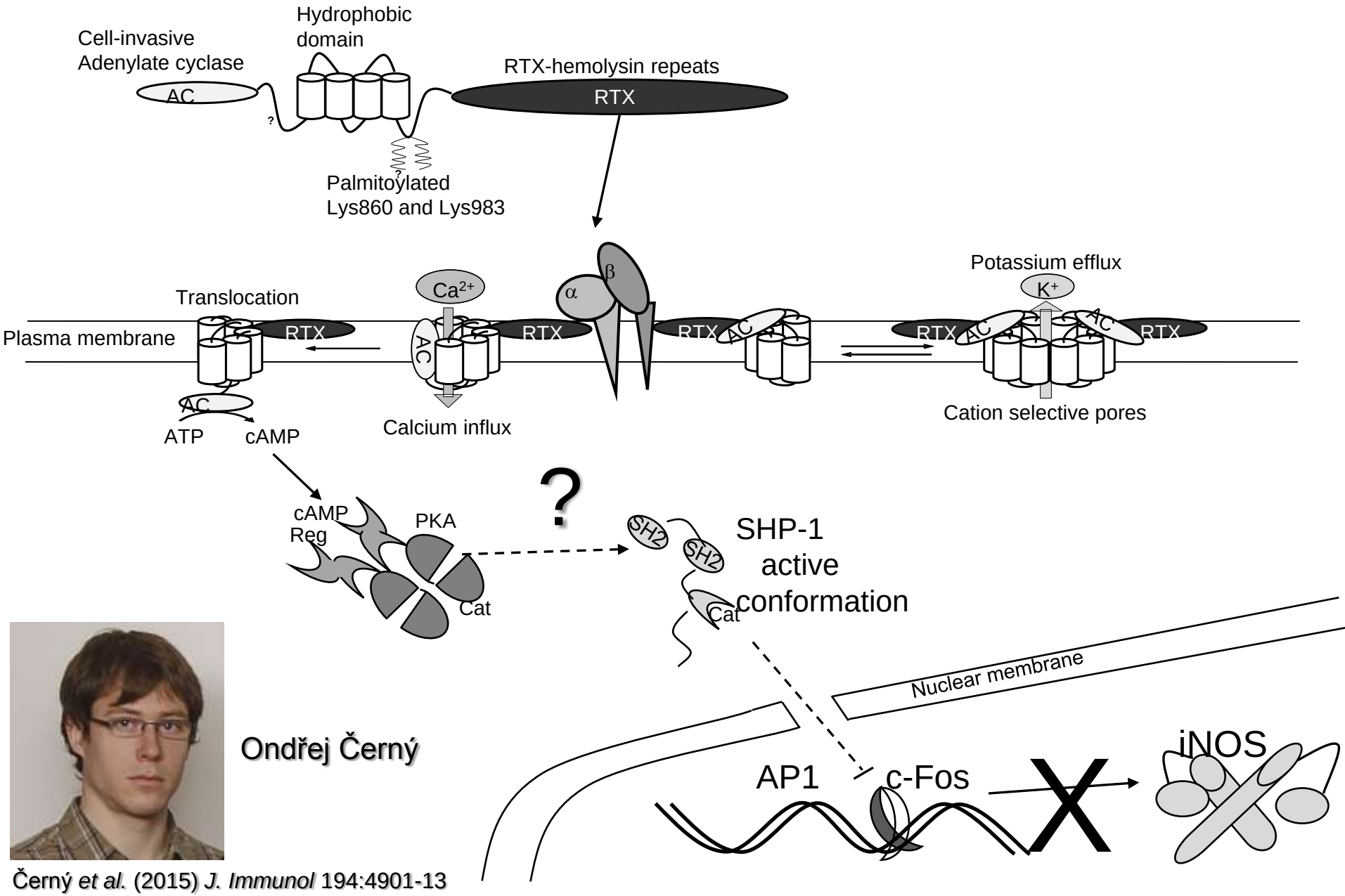






Černý *et al.* (2015) *J. Immunol* 194:4901-13

ACT-produced cAMP signaling through PKA activates SHP-1 tyrosine phosphatase that blocks iNOS gene transcription

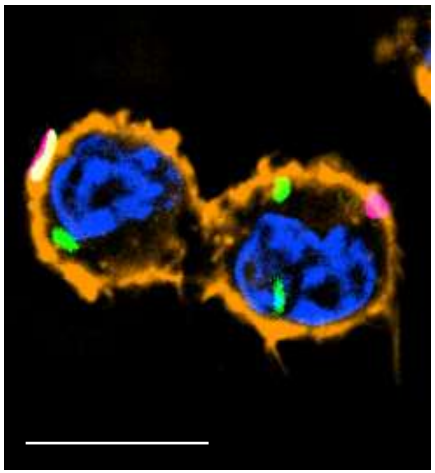


Ondřej Černý

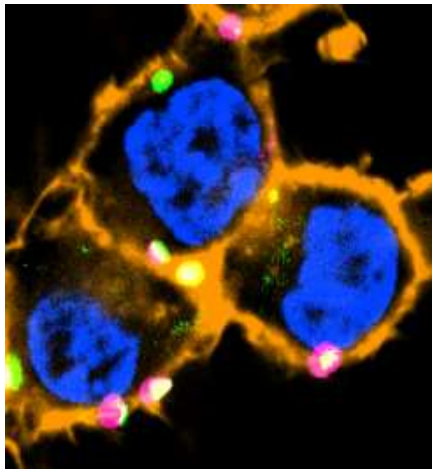
Černý *et al.* (2015) *J. Immunol* 194:4901-13

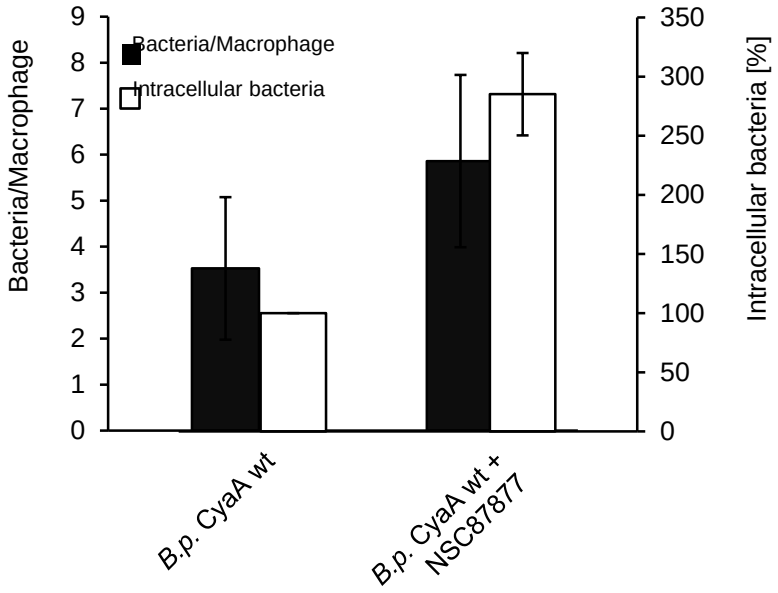
CyaA/cAMP-triggered SHP-1 activation extends *B. pertussis* survival inside macrophages

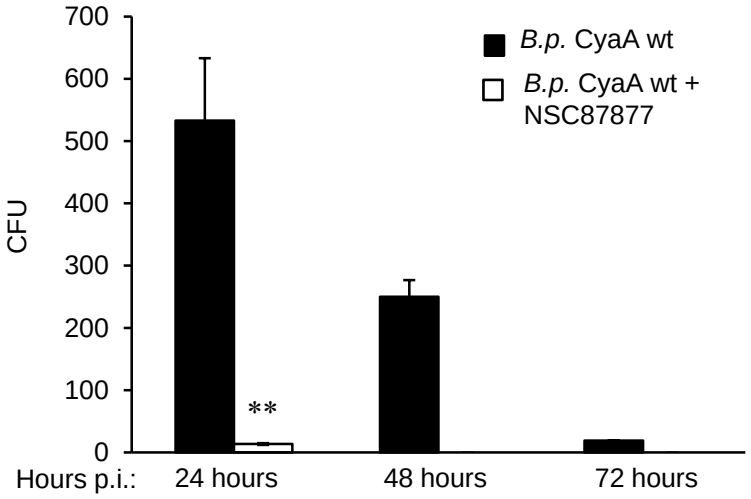
B.p. CyaA wt



B.p. CyaA wt + NSC87877



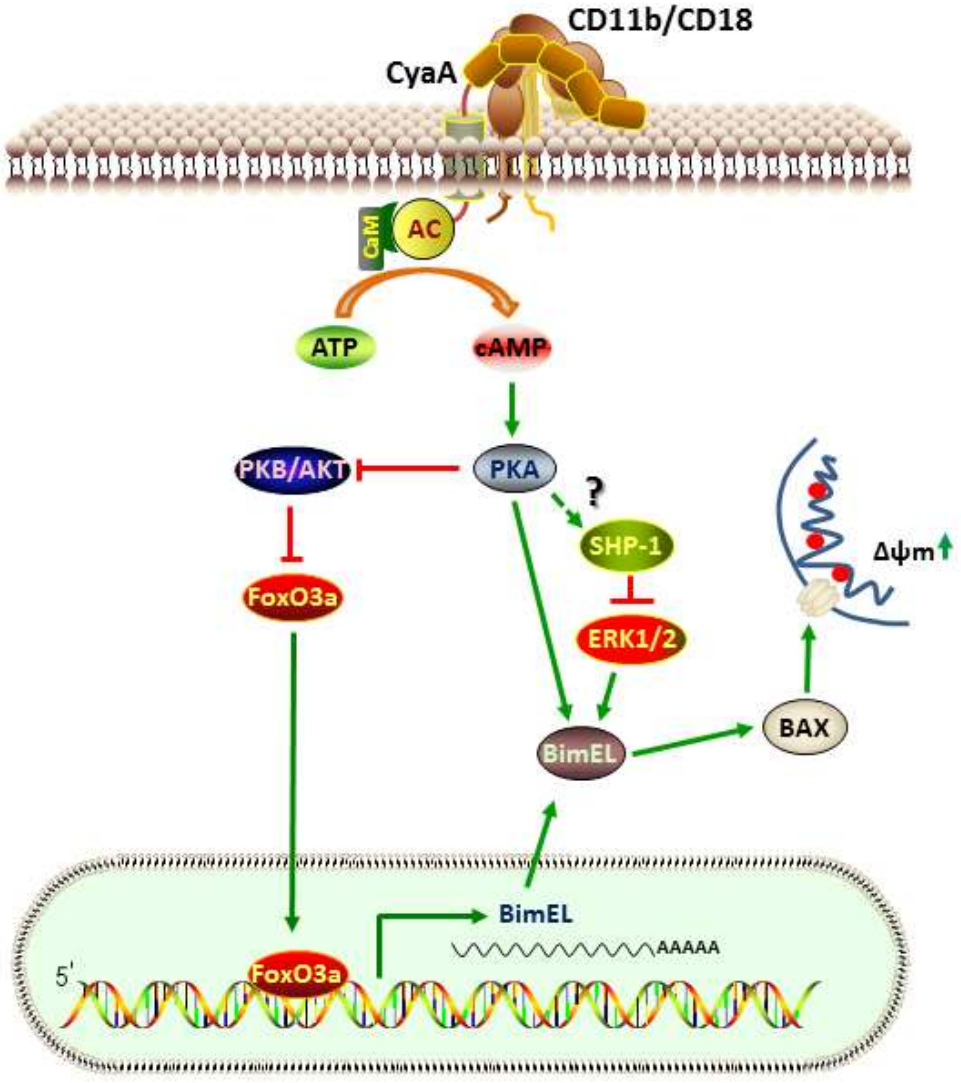




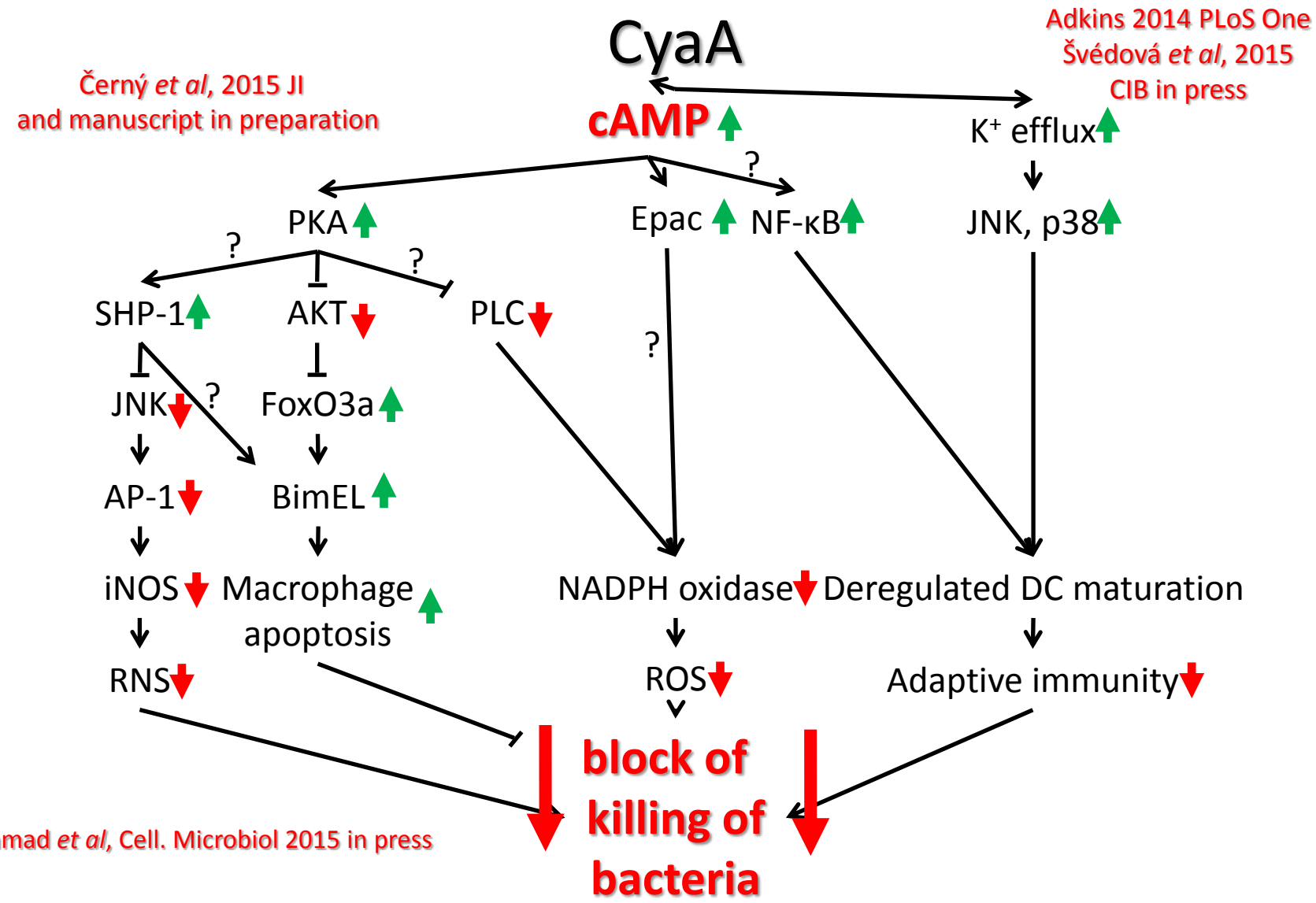
Černý *et al.* (2015) *J. Immunol* 194:4901-13

CyaA/cAMP-triggered SHP-1 activation causes BimEL stabilization, Bax activation and macrophage apoptosis

Ahmad et al. 2015, Cell. Microbiol, in press

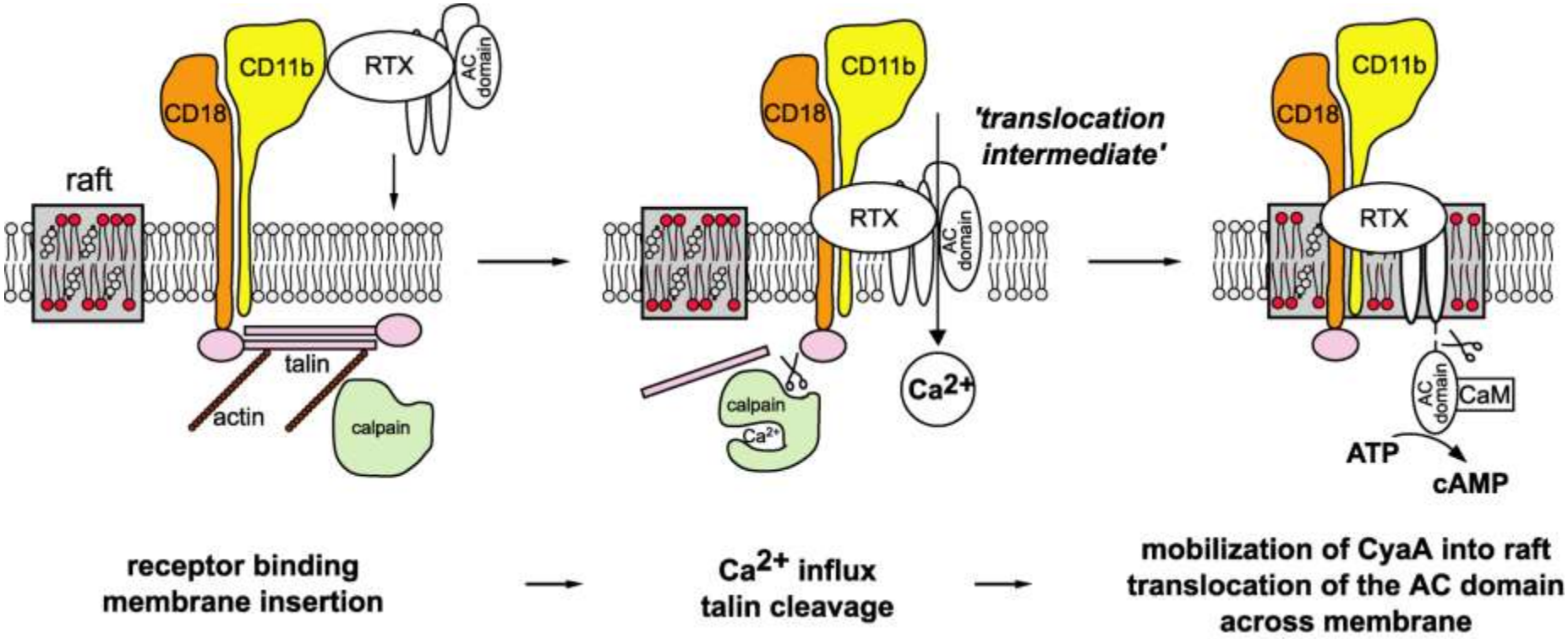


Signalling pathways influenced by CyaA



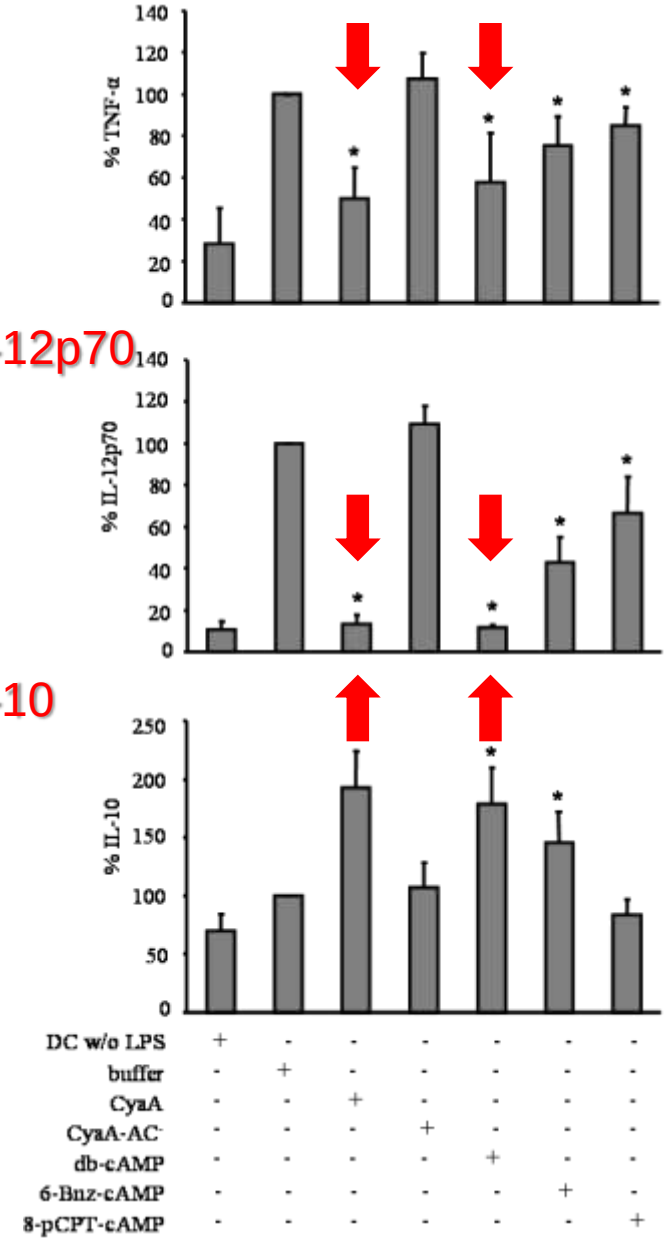
Suppressing adaptive immunity

*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.

TNFα



IL-12p70

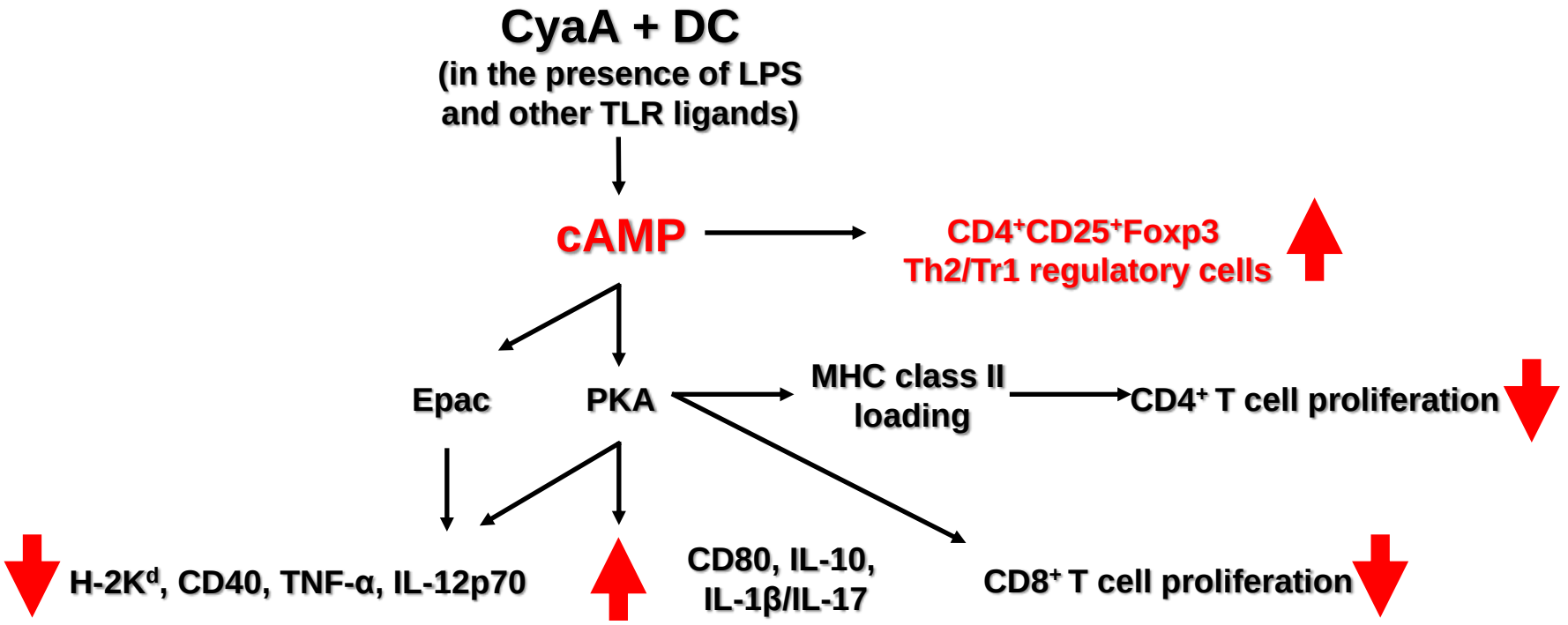
IL-10

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?



Several reports indicated that CyaA subverts adaptive T cell immune reposnes

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

Adkins et al. (2014) PLoS One 9(8):e104064

Hence:

The **cAMP signaling of ACT,**

prevails and

knocks down the innate immunity

and

dampens the adaptive immune response

in order

to enable host colonization by *Bordetellae*

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 …

To sum up this talk:

Rise in pertussis incidence in the most developed countries will likely not stop until the **adenylate cyclase toxoid** has been added to the aP vaccine…

And a less reactogenic nextgen wP vaccine is used as booster…

Sebolab = a PI Confederation…

Radim Osička – CR3 interactions and cAMP signaling of ACT

Irena Adkins – ACT and fooling od DC maturation and cAMP-mediated inhibition of T cell immune response

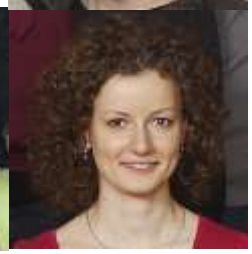
Jiří Mašín – membrane penetration and pore-forming activity of ACT

Peter Šebo – strategic steering, mentoring and paper editing, signaling of cAMP and phagocyte action

Láďa Bumba – structure, folding and secretion of ACT

Ondřej Staněk novel antigen delivery tools Veterinary vaccines

Branko Večerek – sRNA regulation of *Bordetella virulence*



Jana Holubová + **Karolína Škopová** novel pertussis whole cell vaccine

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