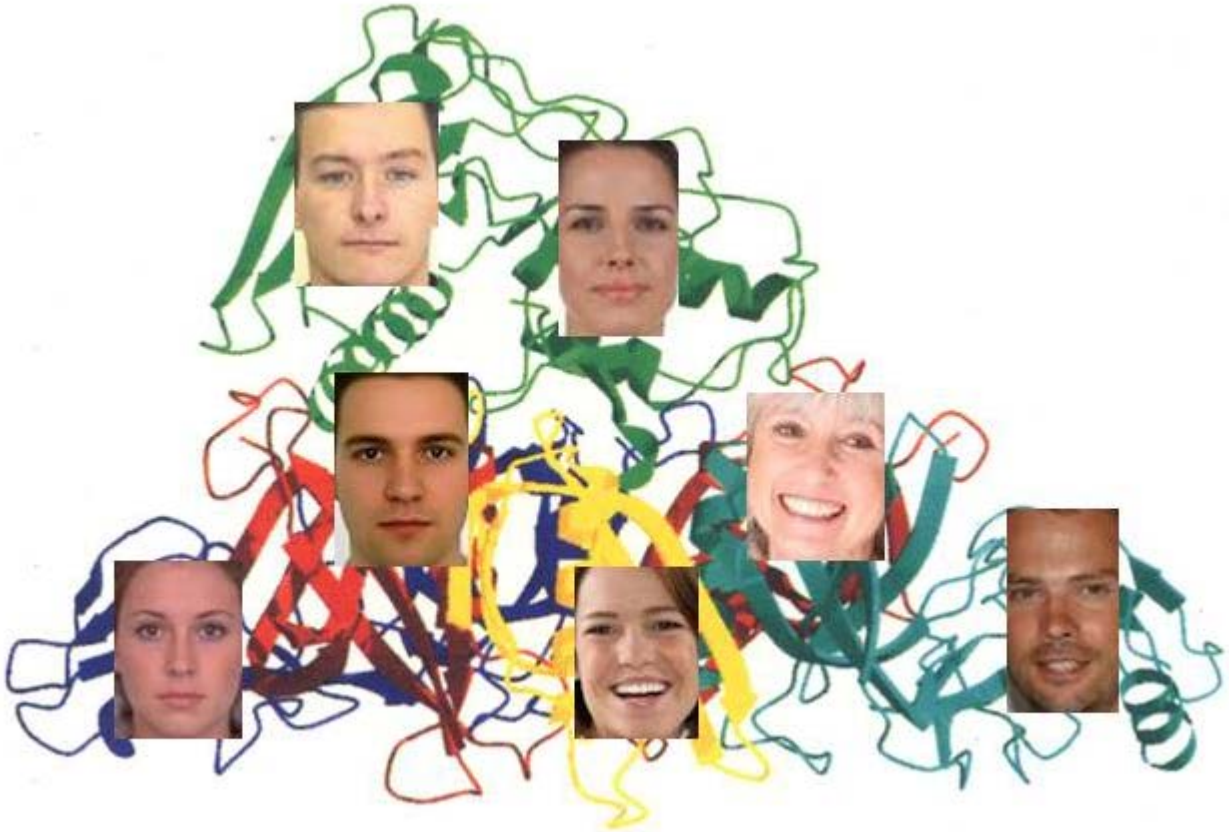
The Many Faces of Pertussis Toxin

Nicholas Carbonetti, PhD

University of Maryland Medical School

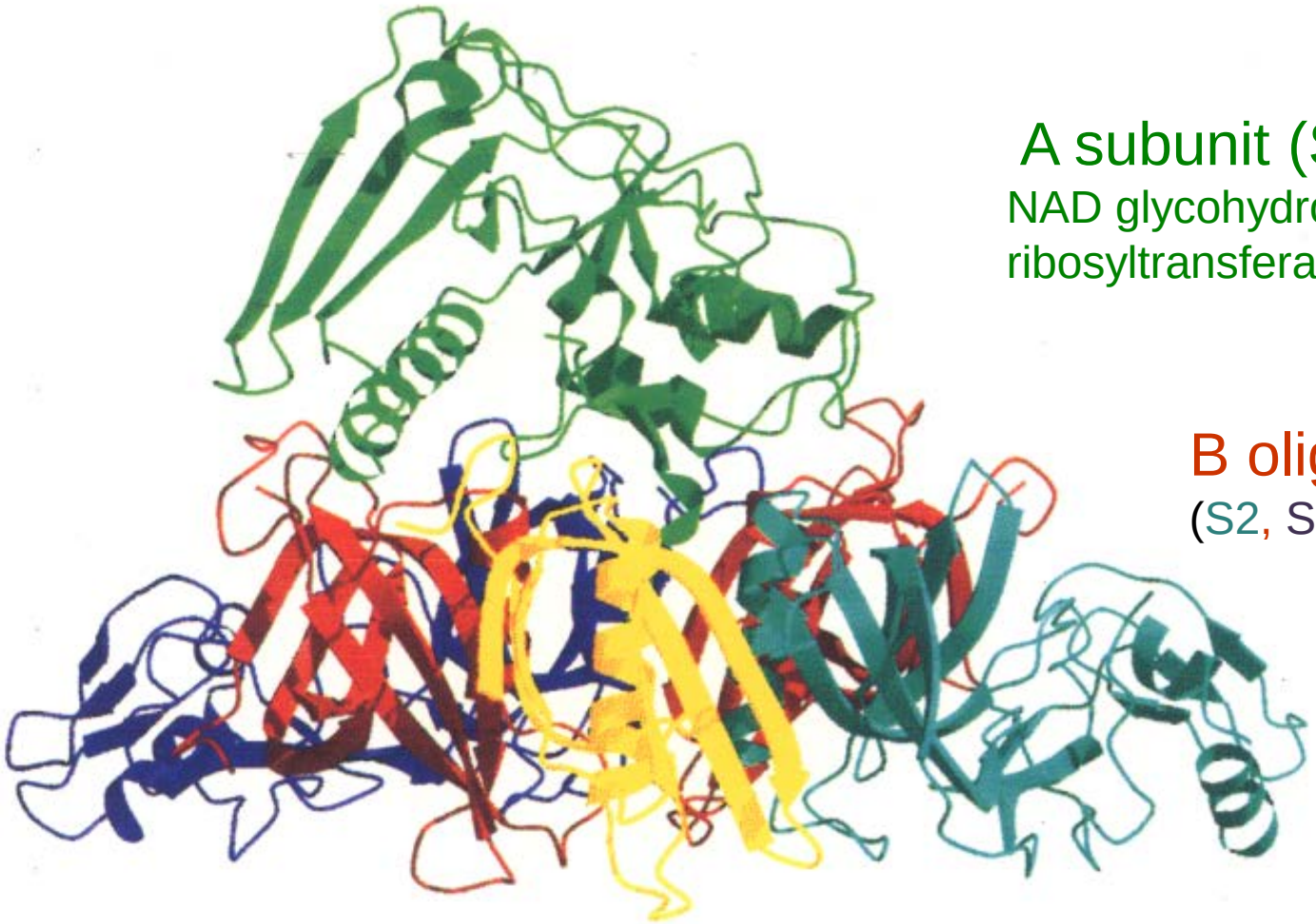


NICEATM-ICCVAM HIST Workshop, November 2012

Historical names

- Histamine Sensitizing Factor (1950s)
- Lymphocytosis Promoting Factor (1960s)
- Islet Activating Protein (1970s)
- Pertussigen (1970s)
- Pertussis Toxin (1980s)
  - No mention of other terms in PubMed title since early 1990s

Pertussis Toxin



A subunit (S1)

NAD glycohydrolase and ADP-ribosyltransferase

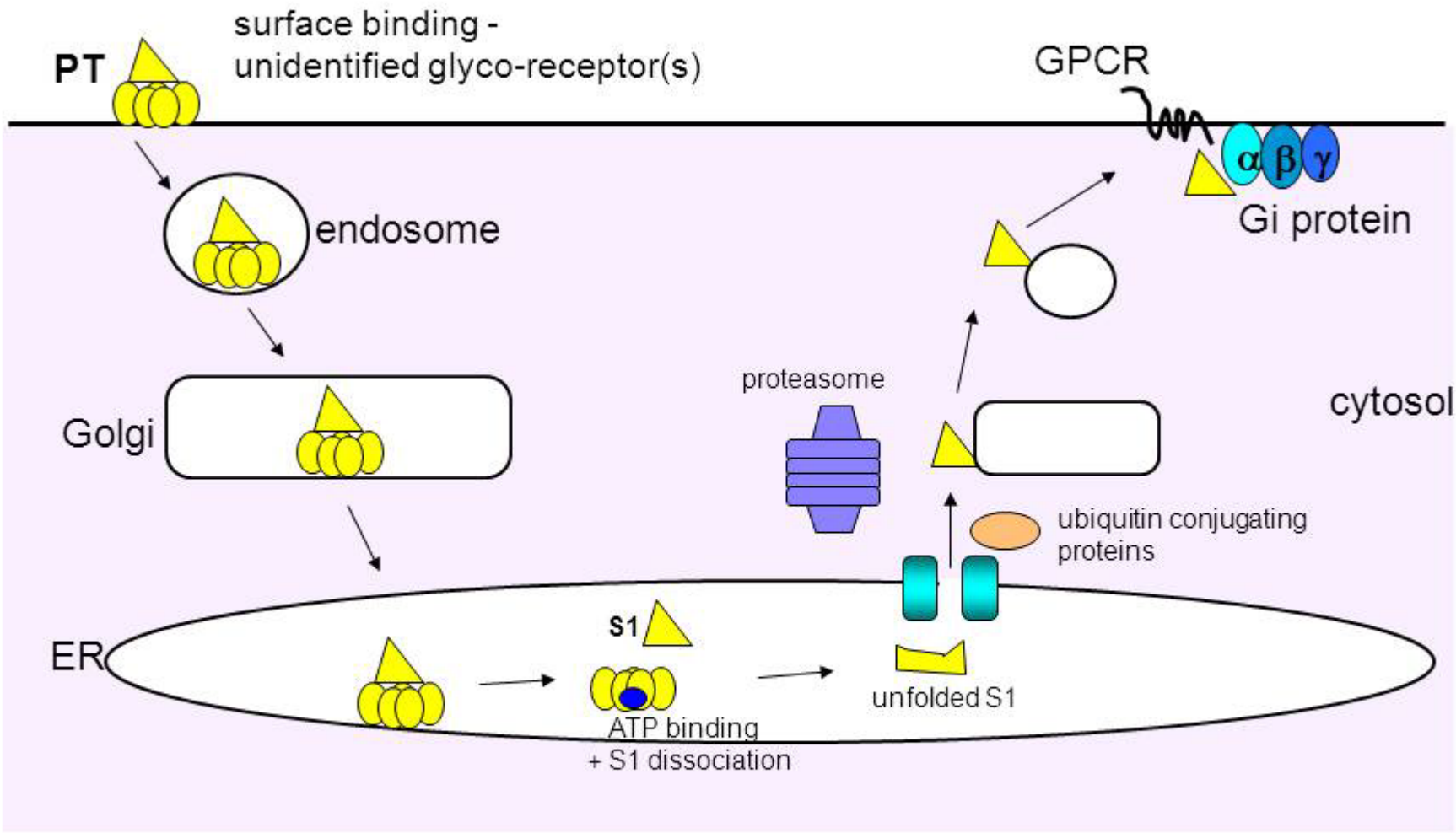
B oligomer

(S2, S3, S4, S4, S5)

AB_5 exotoxin produced uniquely (?) by *B. pertussis*

Stein *et al* (1994) Structure 2:45

Model for intracellular transport of PT

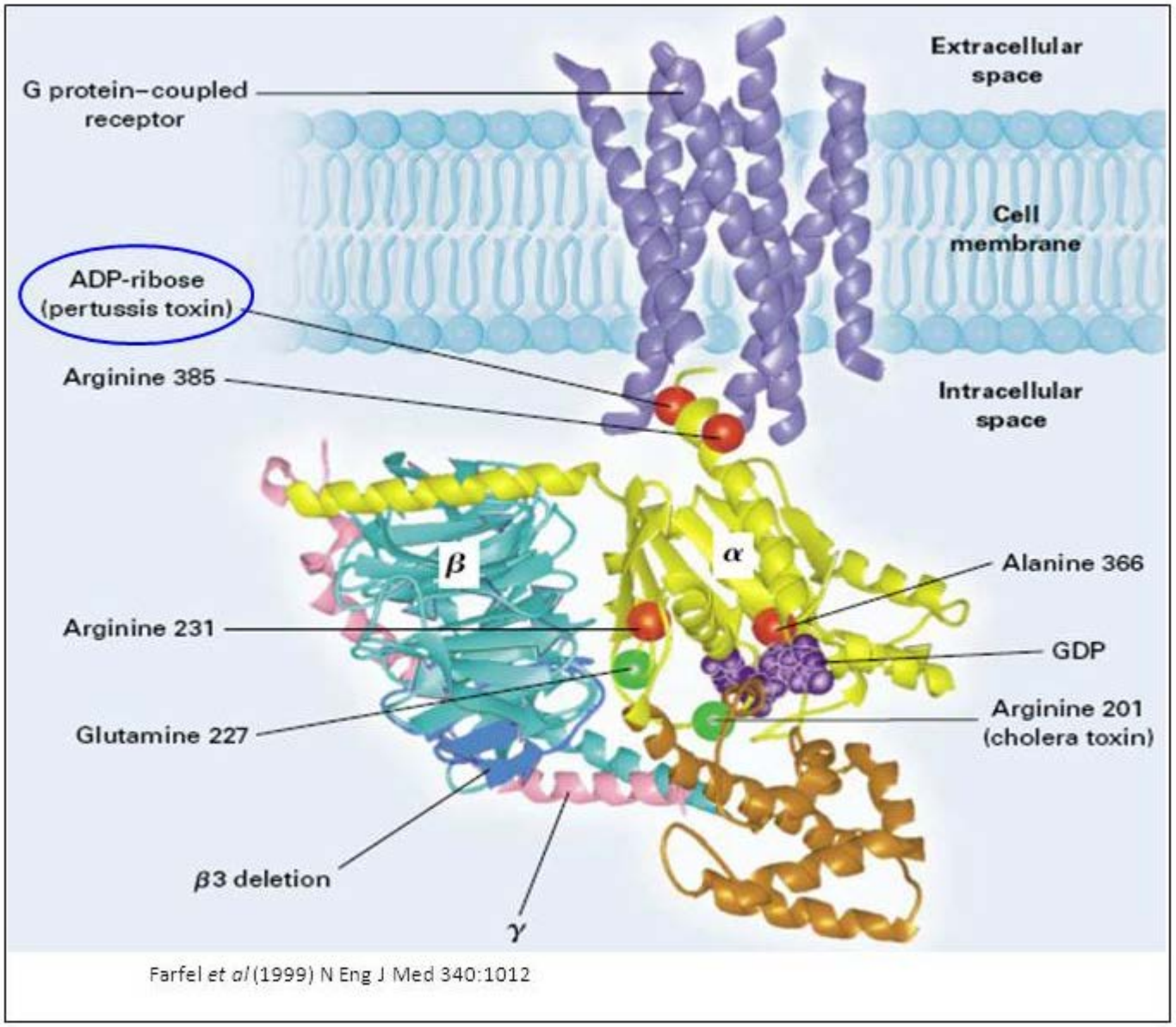


Worthington, ZEV, and Carbonetti, NH (2007) *Infection & Immunity* 75:2946-2953

Plaut, RD, and Carbonetti, NH (2008) *Cellular Microbiology* 10:1130-1139

Plaut, RD, and Carbonetti, NH, unpublished

ADP-ribosylation of Gi proteins by PT



Farfel *et al* (1999) N Eng J Med 340:1012

Activities and Effects of Purified Pertussis Toxin

Inhibits retinoic acid-induced expression of tissue transglutaminase in macrophages

Inhibits yeast phagocytosis by mouse peritoneal and human pulmonary alveolar macrophages

Inhibits leukotriene B4 stimulation of phosphatidylinositol turnover in macrophages

Inhibits B cell and macrophage responses to bacterial lipopolysaccharide

Enhances antigen-specific production in vitro of lymphokine that stimulates macrophage procoagulant activity and plasminogen activator

Stimulates prostaglandin E2 synthesis in a murine macrophage cell line

Inhibits interleukin 3 and colony-stimulating factor 1-stimulated marrow cell proliferation

Reduces macrophage number in bronchoalveolar lavage fluids

Inhibits neutrophil-directed biologic actions of GM-CSF

Inhibits neutrophil synthesis of 5-lipoxygenase induced by arachidonic acid

Inhibits platelet-activating factor release by human neutrophils

Attenuates the development of high blood pressure in spontaneously hypertensive rats

Inhibits IgE-dependent stimulation of macrophages

Inhibits MCP-1 activation of mature human basophils

Inhibits potassium conductance in murine macrophages

Induces nitric oxide production in mouse spleen cells via gamma interferon

Inhibits MIP-1 alpha stimulation of calcium mobilization in neutrophils

Inhibits phagocytosis by Kupffer cells with dysfunction of the actomyosin system

Inhibits myeloid cell proliferation stimulated by Steel factor

Alters mononuclear phagocyte circulation and response to inflammation

Inhibits LDL suppression of NF kappa B activation in macrophages

Inhibits eotaxin induction of oxygen radical production, Ca(2+)-mobilization, actin reorganization, and CD11b upregulation in human eosinophils

Inhibits tissue factor expression in LPS-stimulated bovine alveolar macrophages

Alters innate and adaptive immune responses in a pertussis-dependent model of autoimmunity in mice

Potentiates Th1 and Th2 responses to co-injected antigen

Enhances regulatory cytokine production and expression of co-stimulatory molecules B7-1, B7-2 and CD28

Induces release of inflammatory cytokines and dendritic cell activation in whole blood

Deactivates CC chemokine receptor 5 and blocks entry of M-tropic HIV-1 strains

Induces hyperacute autoimmune encephalomyelitis in Lewis rats

Inhibits phagocytosis but stimulates recycling from phagosomes

Suppresses up-regulation of epithelial ICAM-1 expression

Inhibits voltage-independent Ca(2+) channel modulation by 5-HT in neurons

Inhibits mutant presenilin 2 induction of neuronal cytotoxicity

Abolishes nucleotide inhibition of cyclic AMP synthesis in PC12 cells

Blocks neurotransmitter modulation of K channel activity in neurons

Prevents muscarinic-cholinergic inhibition of cardiac beta-adrenergic inotropic responses

Inhibits acetylcholine-induced contractions of rabbit pulmonary artery

Counteracts intramembrane interactions between neuropeptide Y receptors and alpha 2-adrenoceptors

Abolishes insulin-like growth factor (IGF)-1-induced MAP kinase activation

Blocks lysophosphatidic acid-stimulated inhibition of PTP1B activity

Causes acute stress-induced hyperinsulinemia in rats

Prevents inhibition of glycogen synthesis by EGF

Enhances H2 receptor-mediated action of histamine on hepatocytes

Increases accumulation of cAMP in response to epinephrine in hepatocytes

Abolishes IL2-mediated repression of myocyte contraction via the cardiac kappa opioid receptor

Reduces viral load in SIV-infected macaques

Causes lymphocytosis

Causes rounding and clustering of Chinese hamster ovary cell

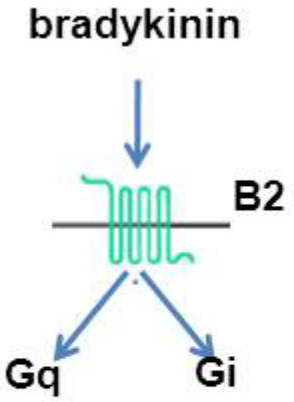
ADP-ribosylation-dependent activities of PT

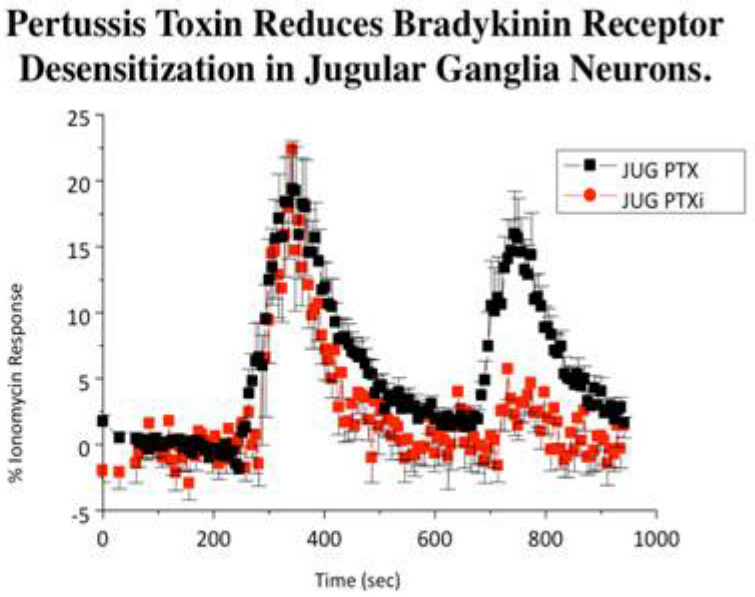
- Increase in cAMP levels
- Inhibition of K^+ channel activation
- Increase in voltage-gated Ca^{2+} channel activity
- Inhibition/activation of MAP kinase activity
- Inhibition of cell migration/chemotaxis

- Histamine sensitization
- Lymphocytosis
- Insulinemia/hypoglycemia
- Vascular permeability changes
- Exacerbation of EAE (?)
- Enhancement of *B. pertussis* respiratory infection and disease!

Possible molecular mechanisms of PT-mediated histamine sensitization

- Mediated via H1 receptor (Vleeming et al BJP 2000)
  - But H1 couples with Gq
  - Switch to Gi coupling to desensitize H1 receptor?





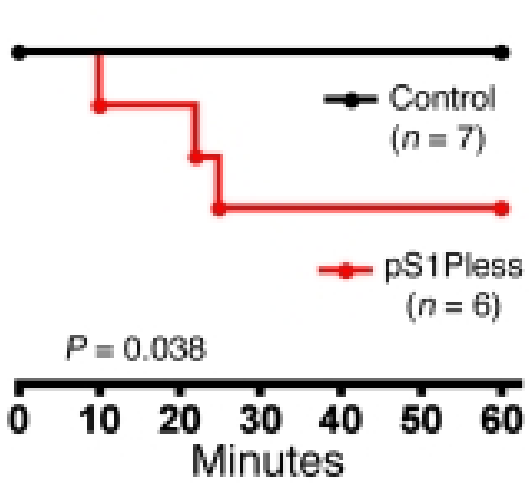
Brendan Canning, JHU

Possible molecular mechanisms of PT-mediated histamine sensitization

- H3 or H4 receptor regulation of H1 signaling
  - H3 and H4 couple to Gi

- Other regulatory Gi-coupled GPCR involved
  – S1P receptor (Camerer et al. JCI 119, 2009)

    • Increased vascular leak after histamine treatment in mice lacking plasma S1P



ADP-ribosylation-independent activities of PT (?)

- T cell mitogenicity
- T cell receptor (TCR) binding and activation
- Adjuvanticity (via TLR4?)
- Cell aggregation
- Endothelial cell ERK activation
- Inhibition of HIV entry and replication
- Cell surface receptor expression changes
  - CXCR4 downregulation
  - Adenosine A1 receptor upregulation
  - Angiotensin type I receptor upregulation

ADP-ribosylation-independent activities of PT (?)

- **BUT**
  - B oligomer activities generally require higher concentrations of PT than those needed for ADP-ribosylation
  - Studies with purified B oligomer potentially complicated by contamination with low levels of active PT
  - Potentially complicated by contamination of purified PT samples with LPS (Bache et al, Med Microbiol Immunol, 2012)
  - Need LPS-free PT-9K/129G !

Other applications of PT

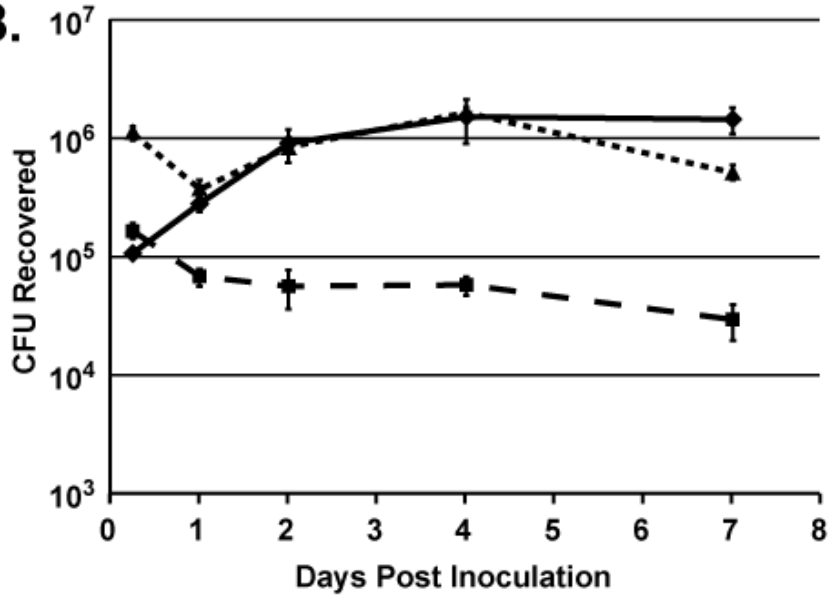
- Acellular pertussis vaccines
  - Common component (detoxified)
  - Only component for some vaccines
- Pertussis diagnosis
  - Serodetection of recent pertussis infection
- Cell biology tool
  - Inhibitor of Gi-coupled GPCRs

PT exacerbates and prolongs airway inflammation

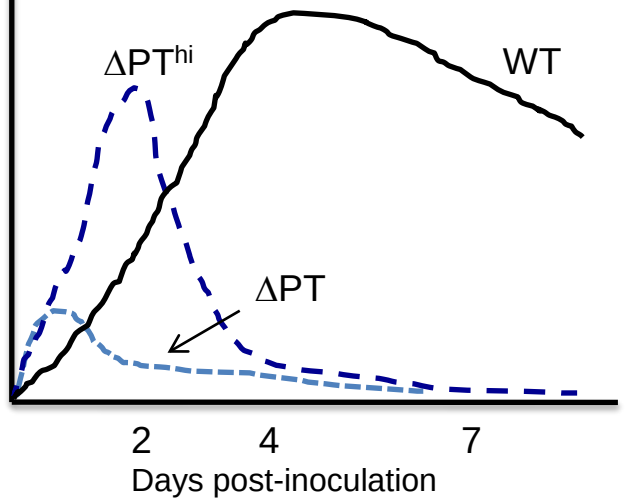
A.

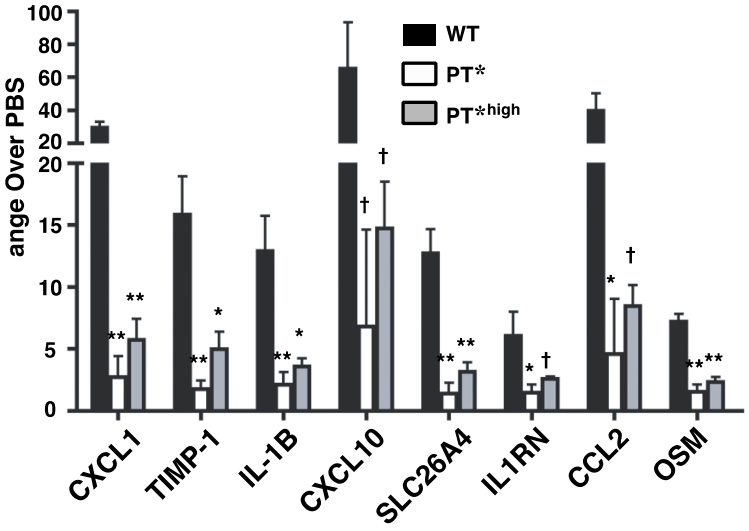
B. pertussis strain	Inoculation dose (cfu)
WT	1.3×10^6
ΔPT	1.3×10^6
ΔPThigh	3.1×10^7

B.



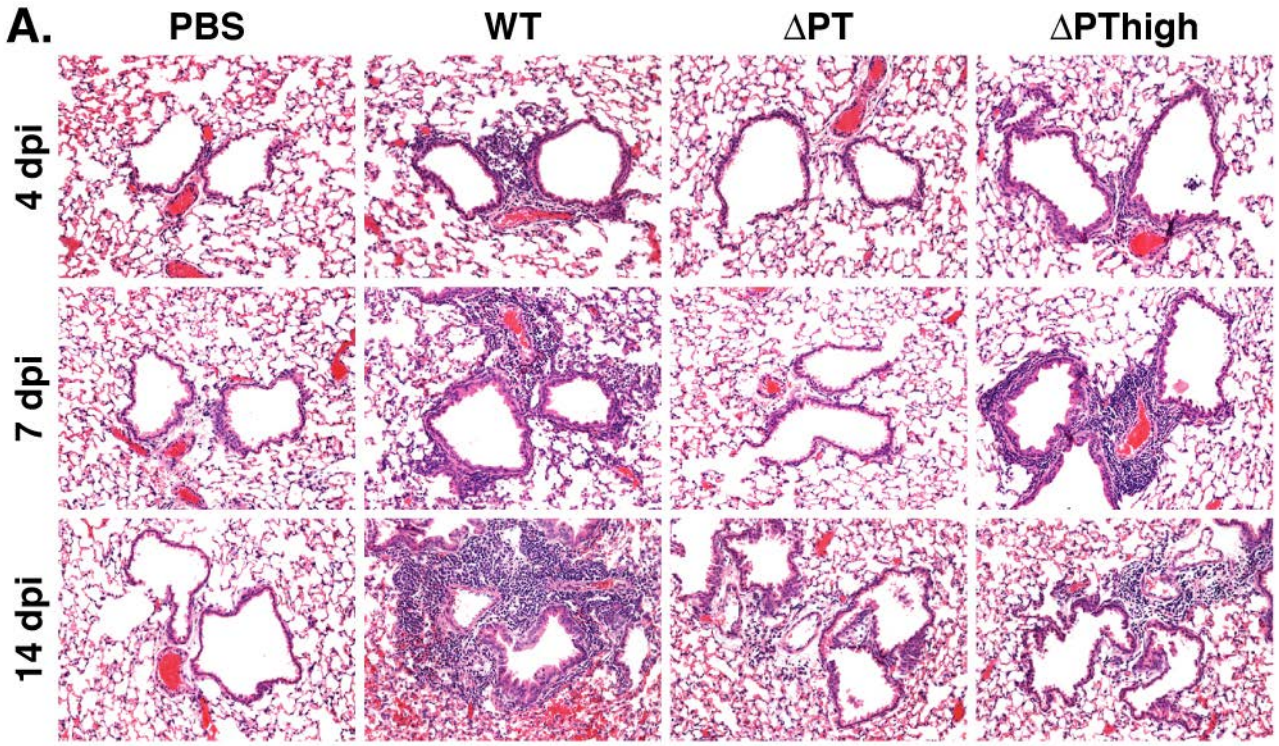
Transcriptional responses

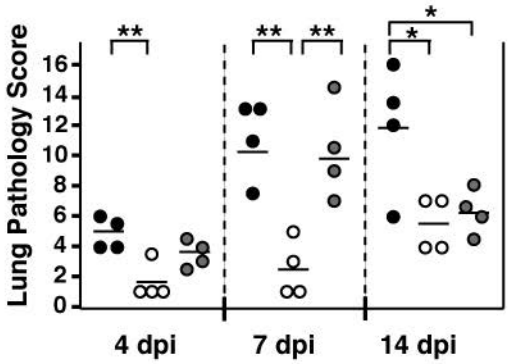


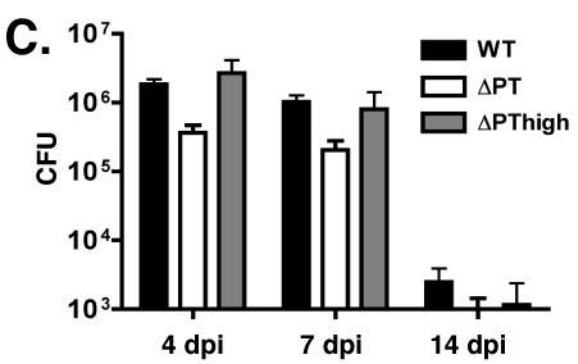


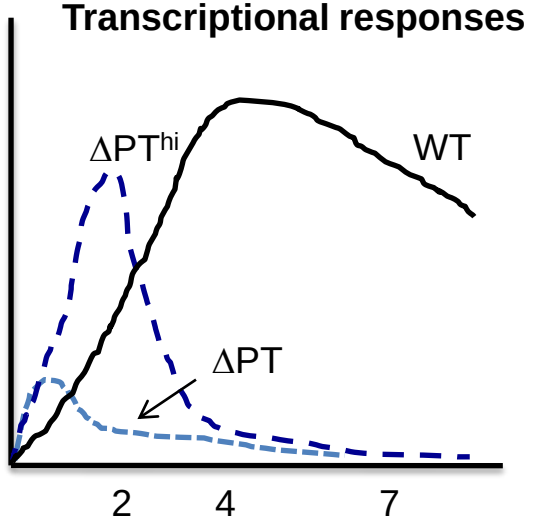
Connelly et al, Infect. Immun. 80:4317, 2012

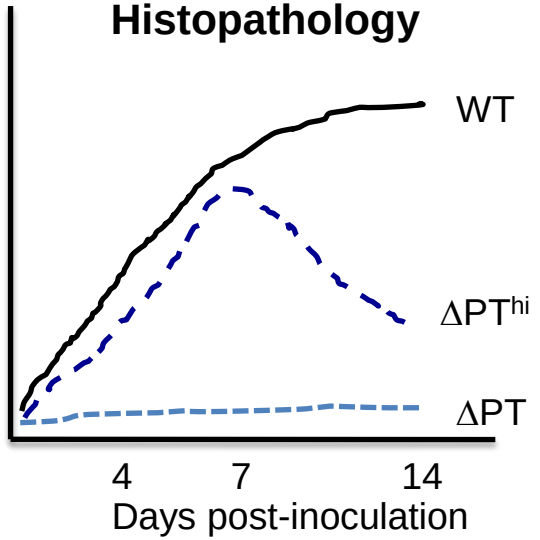
PT exacerbates and prolongs airway inflammation











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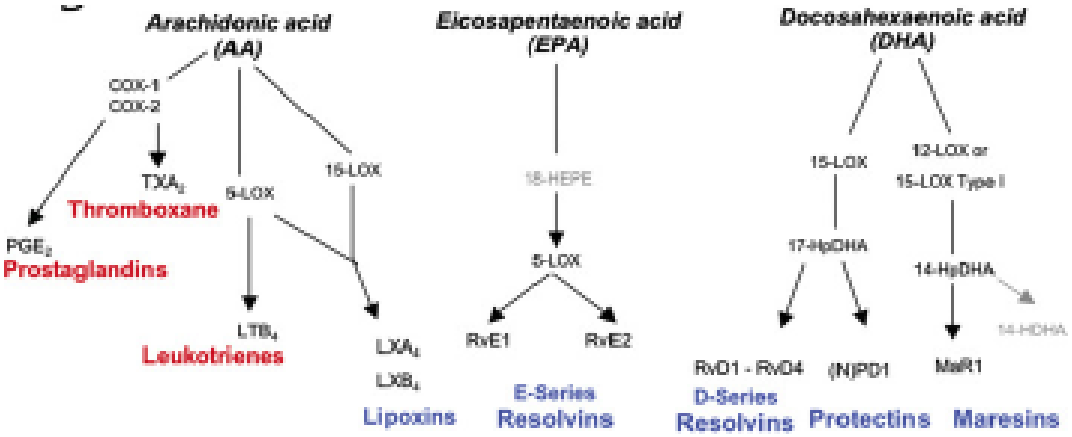
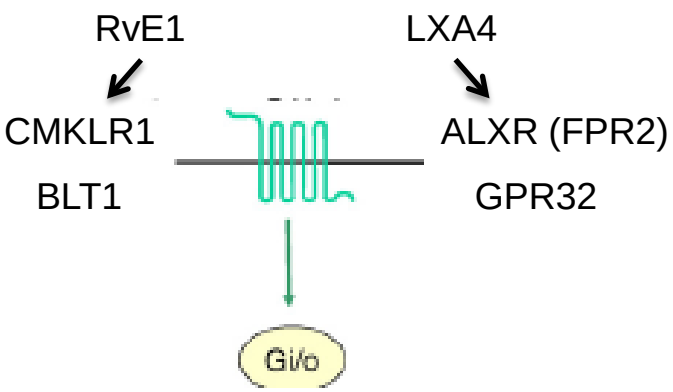
PT exacerbates and prolongs airway inflammation

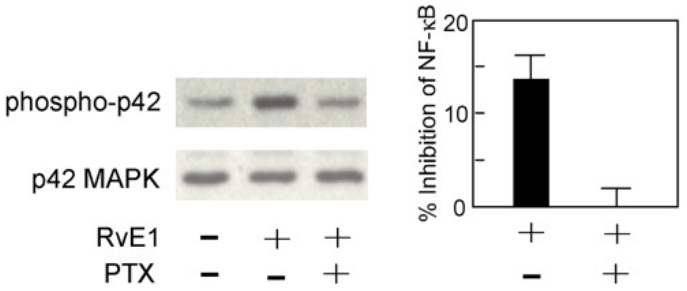
Resolution of Inflammation

Fredman G and Serhan C
Biochem J 437:185 (2011)



Several lipoxins, resolvins and protectins signal through PT-sensitive GPCRs !





Arita M et al. J Exp Med 2005;201:713-722

PT upregulates pendrin expression contributing to airway pathology

Table 3. Top gene expression changes directly related to the presence of pertussis toxin at 4 dpi

		WT vs. PBS		WT vs. ΔPT		WT vs. ΔPT^{high}	
Entrez ID	**Gene**	**FDR**	**FC**	**FDR**	**FC**	**FDR**	**FC**
14825	chemokine (C-X-C motif) ligand 1	0.00	**18.2**	0.00	**18.2**	0.00	**8.0**
16176	interleukin 1 beta	0.00	**11.1**	0.00	**11.0**	0.00	**6.0**
23985	solute carrier family 26, member 4	0.00	**9.4**	0.00	**9.3**	0.00	**5.4**

SLC26A4 = Pendrin (from *Pendred syndrome* – hearing loss, goitre, hypothyroidism)

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