Whoop There It Is:
The Role of Pertussis-toxin in Symptom Longevity

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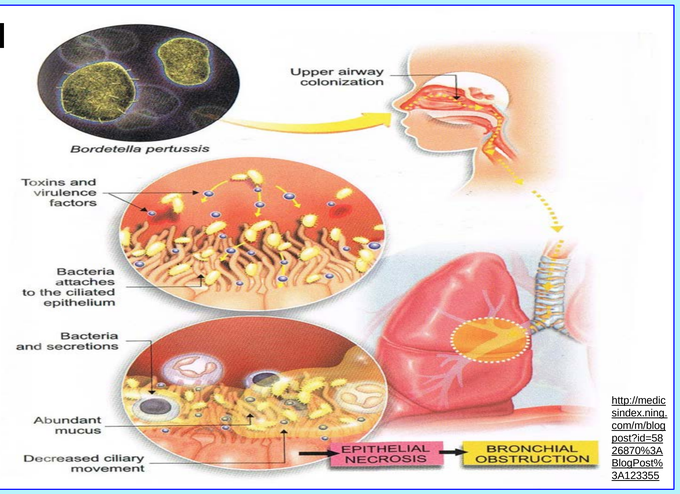
I Abstract

AIDS and Ebola are seen as some of the most frightening diseases on the planet; however, a more infectious disease is on the rise. Pertussis, known as whooping cough, has over a 90% transmission rate between members of the same household. More frightening than its virulence and preventability is its role in infant mortality. Pertussis is a respiratory disease that causes a violently uncontrollable cough and breathing difficulty. Even with immunization, over 48.5 million infections occurred in 2012, with infants accounting for 83% of deaths. Pertussis is caused by a species of bacteria called *Bordetella pertussis* (Bp), which is an airborne, aerobic coccobacillus pathogenic only to humans. Bp infects humans by colonizing respiratory epithelium and producing tracheal cytotoxin, which prevents the cilia from clearing debris from the respiratory tract. Bp then produces pertussis-toxin (PT), which binds to a cell-membrane receptor. The Milwaukee Academy of Science SMART (Students Modeling A Research Topic) Team modeled the pertussis-toxin using 3D printing technology. PT is an AB5 toxin, and exhibits ADP-ribosyltransferase activity. The toxin is endocytosed into respiratory epithelia and traffics through the endocytic pathway through the Golgi complex into the endoplasmic reticulum. The S1 subunit translocates into the cytosol, where the S1 subunit ADP-ribosylates a Gαi protein. This prevents normal migration of leukocytes to the site of infection. This leukocyte immobility contributes to the longevity of symptoms and overall lethality. A greater understanding of this toxin will inevitably result in a more effective and enduring vaccination protocol than currently implemented.

II Pathogenicity of *Bordetella pertussis*

Bordetella pertussis (Bp) is a Gram-negative bacterium that possesses a shape that is a fusion of coccal (spherical) and bacillus (rod-like) shapes. Bp enters the human body through infected airborne droplets and quickly colonizes the ciliated epithelium of the upper respiratory tract. Bp addition to colonizing this surface, Bp produces filamentous hemagglutinin that binds to and paralyzes the cilia of this epithelium, preventing accumulating mucus, bacteria, and debris from being swept away. This accumulation leads to the release of toxins and the development of a persistent and intense "whooping" cough that is the body's attempt to vacate the upper respiratory tract of this excessive mucus. However, the production of pertussis toxin (PT) truly ensures the longevity of this disease. The uptake of PT within leukocytes prevents the immune system from eliminating Bp from the body and creates symptoms that can last in excess of 100 days.

III



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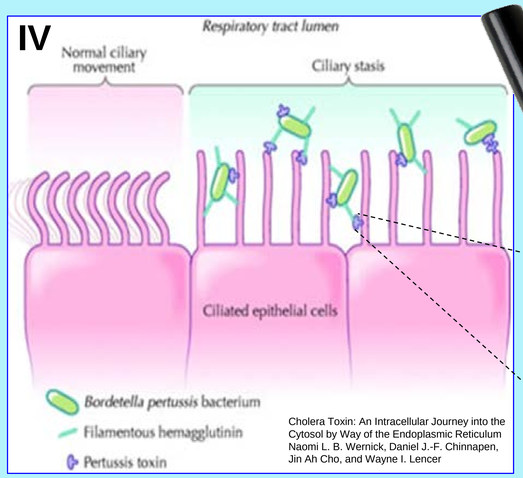

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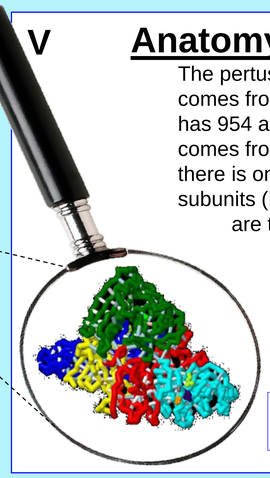
V Anatomy of Pertussis-toxin

The pertussis-toxin is an ADP-ribosylating toxin that comes from the bacteria *Bordetella pertussis*. This toxin has 954 amino acid residues. The pertussis-toxin comes from a family of AB5 toxins. That means that there is one subunit (A) at the head, and five other subunits (B5) in a ring adjacent to the A subunit. There are two S4 subunits. The five subunits have the job of carrying the first subunit throughout the cell. The first subunit does the action of ADP-ribosylating the Gαi protein. The active site of S1 is Glutamic acid 129. This is the part of A that actually does the ADP-ribosylating.

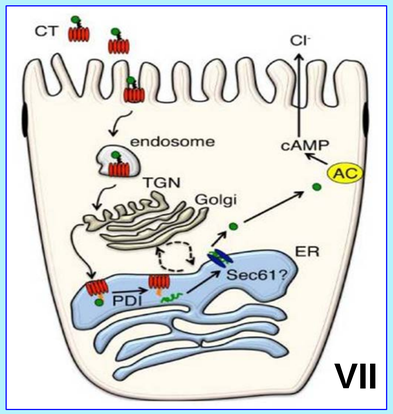


Pertussis-toxin has one A-subunit (S1) that sits atop five adjoined B-subunits (S2, S3, S4, S4, S5)

VI Endocytic Pathway of the Pertussis-toxin

The pertussis-toxin is a product of the *Bordetella pertussis (Bp)* bacterium made up of an A-subunit (S1) that sits atop five adjoined B-subunits (S2,S3,S4,S4, & S5). Upon its release from the *Bp*, the pertussis-toxin attaches itself to a cell membrane receptor. It is then carried by an endosome through the Golgi Complex and translocates it into the Endoplasmic Reticulum (ER). Inside the ER, the A-subunit breaks off from the five B-subunits, unravels, and enters back into the cytosol. This is where the Gαi protein is catalyzed, which increases the levels of cAMP. This is how Pertussis (Whooping Cough) is created.

VII



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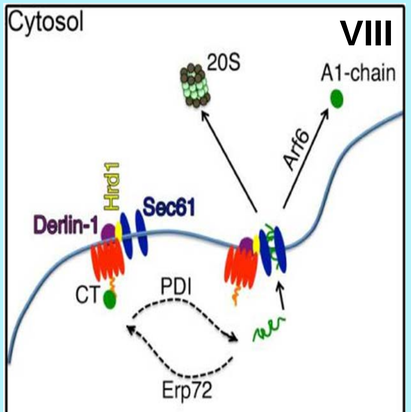

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IX ADP-Ribosylation

ADP-ribosylation is when one or more ADP-ribose molecules are added to a protein. ADP-ribosylation functions include DNA repair, gene regulation, apoptosis, and involvement in many cellular processes like cell signaling. ADP-ribosylation is also the basis for the toxicity, the degree to which a substance can damage an organism, of bacterial compounds such as cholera toxin, enterotoxin, diphtheria toxin, and our protein, pertussis-toxin.

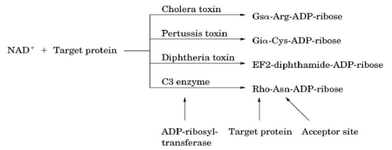


Figure 1. Target protein and acceptor site for ADP-ribosylation in eukaryotic cells by prokaryotic toxins and C3 enzyme.

X PT as Compared to Other Toxins

TABLE I
Sensitivity of CHO cells (K1) to toxins

Toxin	Conc. of toxin (ng/ml) required for morphologic change[a]	
	$-NH_4Cl$	$+NH_4Cl$[b]
DT	3.3	120
PT	0.16	0.16
CT	0.2	0.2

[a] Minimum dose required to induce maximal morphologic changes.
[b] $[NH_4Cl] = 10$ mM.

TABLE III
Release of trapped [^{32}P]NAD from lipid vesicles

Additions	[^{32}P]NAD associated with vesicles[a]
	cpm
None	689 ± 22
PTA	56 ± 8
PTB	537 ± 29

[a] Values represent radioactivity associated with the vesicle fraction determined as described under "Experimental Procedures." Each value is the mean of triplicate determinations ± standard deviation.

Table 1. In this experiment, cells were exposed to one of three toxins (Diphtheria Toxin (DT), Pertussis-toxin (PT), or Cholera Toxin (CT) and then were exposed to varying concentrations of ammonium chloride until the toxin denatured to the point that it no longer retained viability. Due to the similar response to pH PT and CT were proposed to share a common entry pathway into host cells.

Table 3. In this experiment, vesicles were loaded with radioactive NAD and then either filled with the A subunit (PTA), the B subunit (PTB), or neither. The results indicate that PTB and the control group had high radioactive values with the vesicle fraction and that PTA had low radioactive values in comparison. This indicates that PTA is the catalytic subunit within PT.

XI Vaccines

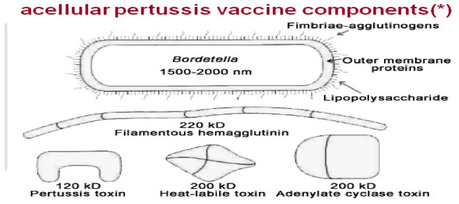
While essential, vaccines,do not provide a lifetime of protection. Vaccinations for pertussis typically offer high levels of protection within the first 2 years of the vaccination but decreases over time, this is known as waning immunity. Before 2005, the only booster available contained protection against tetanus and diphtheria and was recommended for teens and adults every ten years. However, since then a pertussis booster has become more common and many health officials urge people to get them. The vaccines for *Bordetella pertussis* (Whooping cough) are Tdap, common for adults and adolescents, and DTaP, common for children.(D= diphtheria, T= tetanus, A= acellular, P= pertussis) Not only is the vaccine for *Bordetella pertussis*, it also protects you against Diphtheria and Tetanus. The lower case letters mean a reduced dose of that particular portion of the vaccine, as the upper case letters are the full dosage.

- Children should get 5 doses of DTaP at 2, 4, 6, 15-18 months and at age 5.
- Expecting mothers should receive Tdap during their 27-36th week of pregnancy.
- Tdap should be given to children 7-10 years old who have not been fully immunized at younger ages.

Composition* of Acellular Pertussis Vaccines

Product	PT	FHA	PERT	FIM
Daptacel	10	5	3	5
Infanrix	25	25	8	--
Tripedia	23	23	--	--

*mcg per dose



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XII Conclusion

- Vaccine- Children and adults are encouraged to get vaccinated to reduce the chances of getting and spreading Pertussis
- Anatomy- Pertussis toxin is an ADP-ribosylating toxin that has 954 amino acids side chains and has five sub units.
- Endocytic Pathway- The Pertussis toxin attaches itself to a cell membrane, travels through the Golgi Complex, then moves through the Endoplasmic Reticulum. Inside the ER, the A subunit breaks off and enters back into the Cytosol.
- ADP ribosylation-It is also the basis for the toxicity, the degree to which a substance can damage an organism, of bacterial compounds such as cholera toxin, enterotoxin, diphtheria toxin, and our protein, pertussis toxin.

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