Extracellular Pathogens: Clostridium

Tuesday, 3 May 2016 1:07 PM

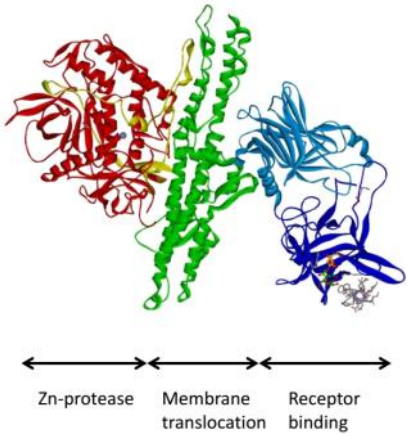
- Be able to describe what type of diseases are botulism and tetanus
- Be able to discuss the mode of transmission
- Be able to explain the molecular mechanisms of pathogenesis
- Be able to differentiate between the mode of action of botulinum and tetanus toxins

Clostridium

- Gram positive anaerobic
- Ubiquitous in environment
- Most are very proficient at forming spores
- Generally produce numerous exotoxins, some of which are virulence associated
    - Bile salts protect us from spore germination

Neurotoxins

- Among most potent toxins known
- Secreted or excreted (exotoxins)
- Encoded in gene clusters on the bacterial chromosome, plasmid or prophage
    - Evidence for horizontal gene transfer independent of core clostridial genome
- Several serotypes of BoNT
- No obvious function apart from killing of animals

- Single precursor polypeptide
- Two subunit mature toxins (localisation and catalysis) - AB toxin
- Catalytic subunits cleave docking protein in synaptic vesicles
- Block synaptic transmission at nerve endings
- Cause botulism (BoNT) and tetanus (TeNT)
- Treated with antisera and supportive therapy (ventilation, nutrition)
- Recovery requires regeneration of nerve endings to recover muscle function
- 

Exam question

Botulism

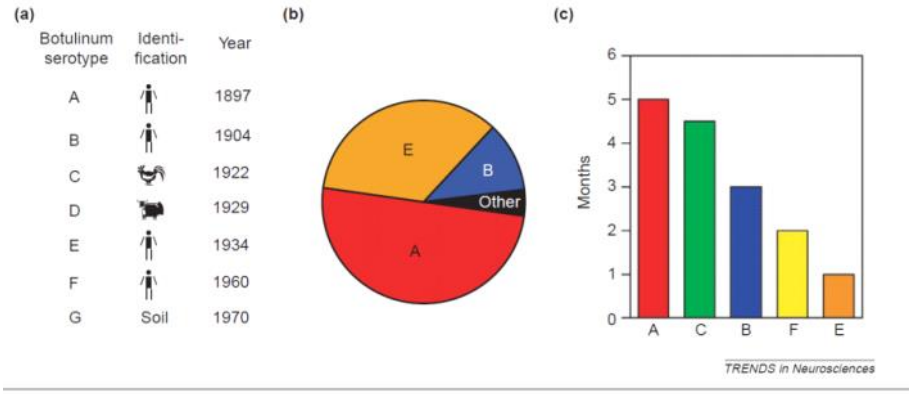
- "Flaccid paralysis"
- Not usually found as an infectious disease, toxins causes damage
- Stored food in which Clostridium botulinum has grown and produced BoNT
- Infant and wound botulism result from infections
    - Babies do not have normal competitive flora, and cannot produce bile salts to inhibit spores

- Median lethal dose about 1ng/kg in mice
  - A few hundred grams could kill all humanity
- BoNT cause flaccid paralysis
- Symptoms include

Metranidazole effective against anaerobes

  - Double vision, dysphagia and dry mouth. It can be followed by descending flaccid paralysis which may be associated with respiratory and cardiac paralysis resulting in death.

- Most cases of infant botulism are in developed countries. Food-borne disease usually from homemade preserves
- Animal botulism must more prevalent

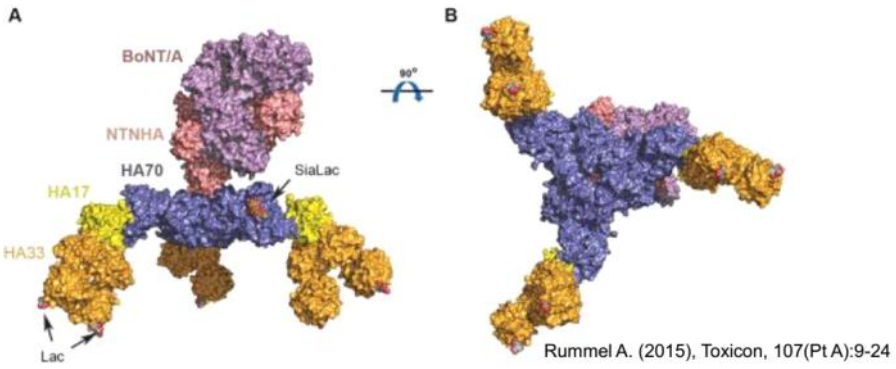


Discovery | Food-borne botulism (USA) | Paralysis length

Serotype: different variation of the protein. Classified and detected by how they react with antibodies, toxins neutralized by different antibodies are different serotypes although variations can exist between serotypes

- BoNT is encoded in complex genetic loci
  - Mobile genetic elements facilitate horizontal gene transfer and recombination of BoNT types
  - Accessory protein: NTNHA
  - Protein undigested due to protease resistant complex
- TeNT encoded in a simple genetic locus

BoNT is secreted as a large complex

- 

Rummel A. (2015), Toxicon, 107(Pt A):9-24

NTNHA and other toxin-associated proteins form a complex with BoNT to stabilise it in the GI tract and promote intestinal absorption

Multiple carbohydrate binding sites differing between complexes

Different effects on intestinal epithelium

- Transcytosis across M-cells
- Disruption of E-cadherin junctions on basolateral side

Tetanus

- "Rigid paralysis" or "lockjaw:
- Descending paralysis
- Infection: C. tetani spores delivered to anaerobic niche in the body
- TeNT produced by growing bacteria
- Treatment like for BoNT plus antibiotics due to live infection
- Symptoms include
  - Mild spams, powerful spams (may cause spinal fractures), drooling, sweating, uncontrolled urination and defecation, respiratory failure and death
  - Case-fatality rate patients ≥ 65 years: 50%; 21-64 years: 13%

Need high calorie diet to be kept alive, muscles contracting constantly burns a huge amount of energy
Up to 5000 calories

Vaccine available, boosted every 10 years

- Efficacious vaccine available (Diptheria, Tetanus, Pertussus)
  - No natural immunity after infection
- Deep wounds with a foreign body require vaccine boost if not up to date

Maternal and neonatal tetanus

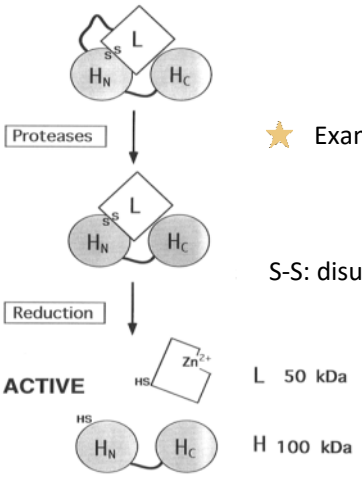
- Neonatal tetanus frequent in developing countries
  - Insufficient maternal immunity
  - Non-sterile cutting of umbilical cord
  - It may present in the first 24 hours of life
- In 1991, WHO estimated around 433,000 newborns deaths due to neonatal tetanus
- Key intervention is maternal immunisation with tetanus toxoid and clean delivery practices
- Maternal mortality rates up to 52% are reported in Asia and Africa. Neonatal mortality ranges from 3% to 88% in these regions.

Pathogenesis of Botulism and Tetanus

- Ingestion or production of neurotoxin
- Binding of toxin to nerve endings
- Translocation of toxin into nerve cells
- Activation of catalytic subunit
- Blockage of neurotransmitter release
  - BoNT at neuromuscular junction
  - TeNT at inhibitory interneurons in motor pathways

Toxin structure

- Botulinum and tetanus toxins are closely related proteins.
- There are seven antigenically distinct botulinum toxins but only one tetanus toxin.
- Synthesised as single polypeptides with three domains.
- Cleaved by proteases and activated by thioredoxin under reducing conditions.
- L (light) chain is catalytic toxic component
- H (heavy) chain is required for uptake of toxin into cell (H_C) and exit from the endocytic vacuole (H_N).

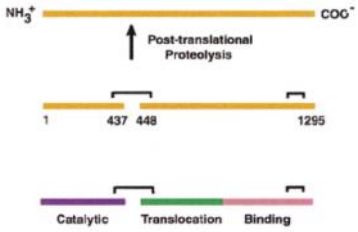


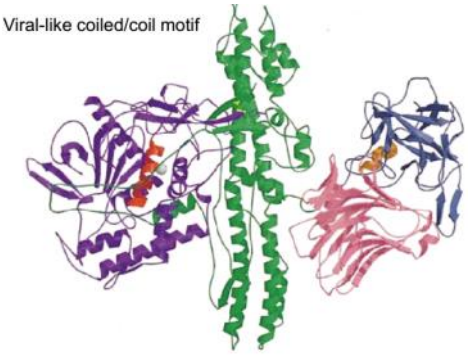
Schiavo & Montecucco, 1997

★ Exam question

S-S: disulfide bridge > reduced

- All proteins with disulfide bridges are extracellular and cleaved in the cytosol
- Active catalytic site is the light chain which is a zinc dependent protease, requires metal atom, preferentially zinc





Lacy & Stevens J Mol Biol 291: 1091-1104, 1999

Purple: catalytic domain
- HExxH Zn-binding motif is **red**
- Zn is grey sphere

Green: translocation domain, transmembrane helix

Pink/Blue: binding domains

1. Binding
2. Endocytosis
3. Translocation.
4. Cleavage.

In the neuron
Outside endocytic vesicles

Model for toxin translocation

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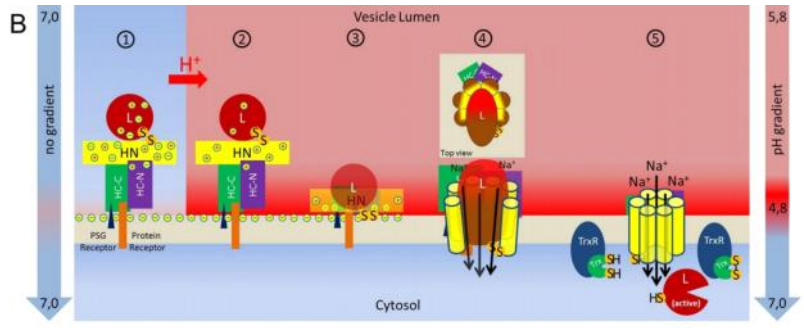
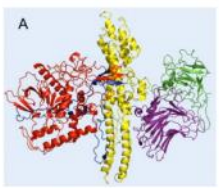
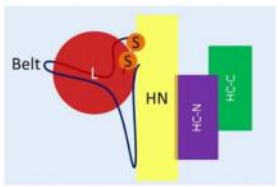


Pirazzini, M. et al (2016), Biochimica et Biophysica Acta (BBA) - Biomembranes, 1858(3):467–474

L: Light chain (catalytic domain)
HN: Amino-terminal domain of heavy chain
HC-C: Carboxy-terminal domain of heavy chain
HC-N: Amino-end of carboxy-terminal domain of heavy chain



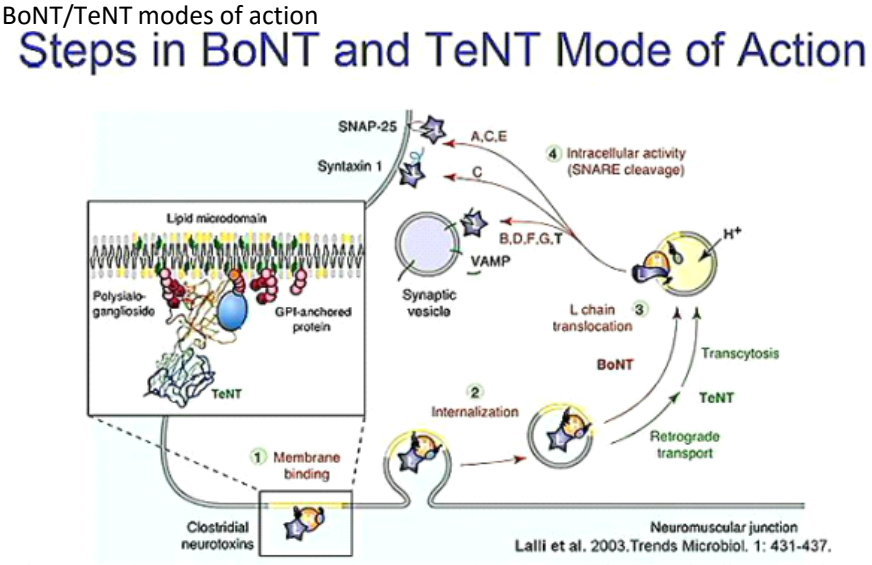


- Heavy chain binds to specific receptors > several. Only binds to presynaptic membrane of motor neurons choosing glycolipids and specific proteins receptors
  - Proteins belong in synaptic vesicles. When vesicle fuses, machinery becomes part of

the presynaptic terminal and is recycled back into the terminal

- Vesicle becomes acifidifed > V-type ATPase. Causes drop in pH, changes conformation of the toxin and collapses onto the membrane
  - Alpha helixes (yellow) reorganise into several smaller alpha helixes to form a pore in the membrane
  - Allows catalytic light chain to be extruded into the cytosol, still attached to membrane by disulfide bond
  - Disulfide bond is reduced by thioredoxin allowing the toxin to enter the cytosol. Thioredoxin has correct redox potential

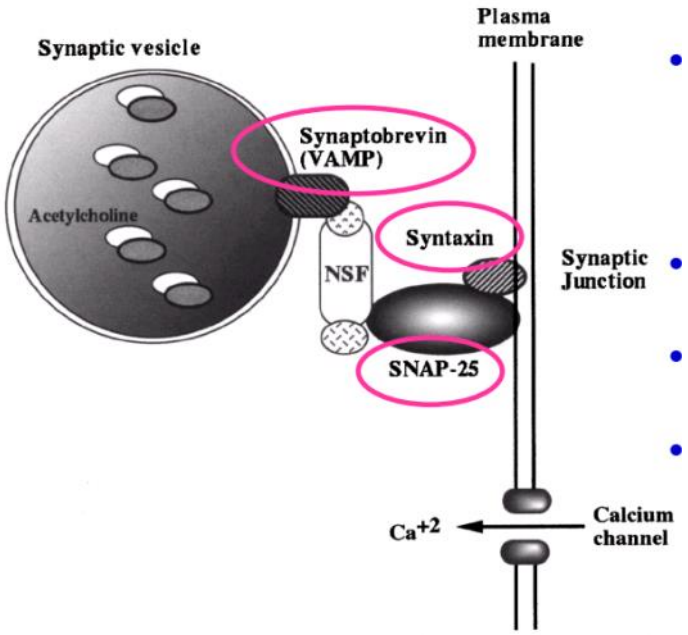
BoNT/TeNT modes of action



1. Binding to receptors on pre-synaptic membrane (glycolipids and SV proteins)
2. Internalisation by SV recycling machinery (vesicle fate differs for BoNT and TeNT)
3. Once vesicle acidifies, conformational change and transport of L chain to cytoplasm
4. Proteolysis of target SNARE proteins

- Vesicles containing TeNT are not acidified, do not have the appropriate markers for machinery recycling > undergo retrograde transport
  - Happens over long distance, axons send things back for recycling to the cell body
- Vesicles containing TeNT engage retrograde transport and are transported back to the CNS where they are transcytosed
  - When they reach the neuronal body, they are exocytosed into the synaptic terminal which happen on the neuronal body
  - Toxin is taken up by next neuron up the chain into a recycling vesicle which is acidified
  - Tetanus toxin does not act on uptake neuron, acts on neuron upstream. For motor neurons, this is in the spinal cord

Synaptic vesicle fusion

- 

- Involves formation of a complex between v-SNAREs on synaptic vesicle ie. VAMP and t-SNAREs ie. SNAP-25 and syntaxin on target membrane
- All members of the complex are necessary for vesicle fusion
- NSF: N-ethylmaleimide sensitive factor
- SNARE: soluble NSF attachment receptor

- TeNT targets
  - VAMP (synaptobrevin)
- BoNT
  - VAMP (synaptobrevin) and/or SNAP-25, syntaxin

Overall

- Light chains of both toxins are Zn-endopeptidases specific for core proteins of the neurotransmitter release apparatus
- All these core proteins (VAMP/synaptobrevin, SNAP-25 and syntaxin 1) are necessary for the fusion of synaptic vesicles (SV) at the nerve terminal and constitute the synaptic members of the SNARE family
- The selective proteolysis of synaptic SNAREs accounts for the total block of neurotransmitter release caused by clostridial neurotoxins.

Differences

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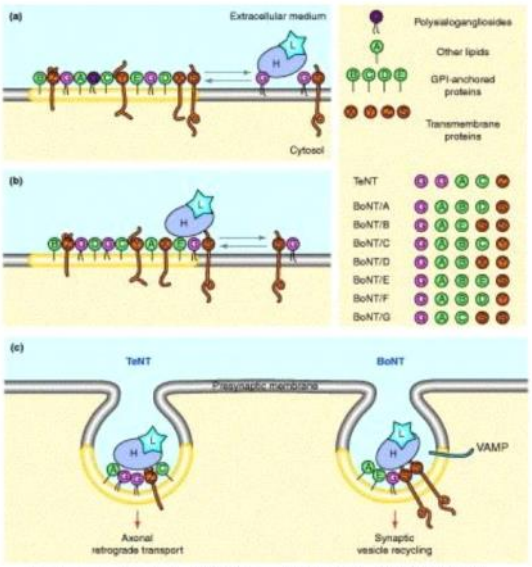
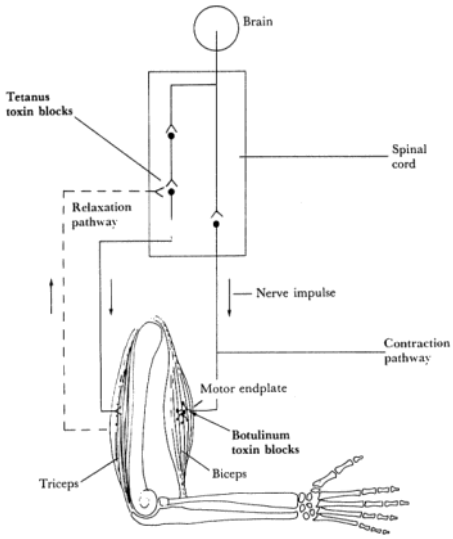


Montecucco, C. et al., (2004), Trends Microbiol., 12(10):442-446

- Heavy chains bind to glcolipids and proteins from SVs in pre-synaptic membrane
- SVs are recycled by endocytosis
- Heavy chains recruit different proteins to endocytic vacuole
- BoNT recruits recycling proteins (V-ATPase)
- TeNT recruits retrograde transport proteins
- BoNT-containing vacuoles are acidified
- TeNT-containing vacuoles are delivered at post-synaptic membrane

- Receptors engaged have different cytoplasmic tails, since tails are different, they are associated with different toxins
  - TeNT: retrograde transport
  - BoNT: recycling

Tetanus toxin blocks the relaxation pathway = rigid paralysis



Botulinum toxin blocks the contraction pathway = flaccid paralysis

Therapeutic uses of botulinum neurotoxins

- Injections of low levels of BoNT/A into striated muscle leads to reversible denervation of neuromuscular junction
- No permanent effect on anatomical contact between nerves and muscles, no loss of motor axons

➢ Facial dystonias
➢ Cosmetic industry
➢ Effects last 1-4 months