Bacterial toxins

Overview of most important bacterial toxins

- Toxic bacterial proteins
  - Staphylococcal enterotoxin B
  - Diphteric toxin
  - Clostridial toxins
  - Botulotoxin
  - Tetanospasmin
  - Shigatoxins
- Toxic lipopolysaccharides
  - O-antigens

Toxic bacterial proteins

- **Gram-positive bacteria**
  - From cells released to environment during the growth of living bacteria (exotoxins).
- **Gram-negative bacteria**
  - After disruption of cell memebrane (endotoxins)

- Molecules with molecular weight 30 000 to 150 000
- Peptidic chains
- Thermolabile (inactivated by boiling)
- Usually good immunogenic properties
  - Activity is blocked by specific neutralization antibodies
    - Could be changed to toxoids
- Way of toxic effect prevalently characteristic
  - Toxinoses
    - Diphteria, tetanus, botulism and cholera
- Production coded:
  - chromosomal (*Vibrio cholerae, Arcanobacterium haemolyticum, Corynebacterium pseudoterberculosis*)
  - at plasmides (enterotoxins of *Escherichia coli*)
  - linked to presence of specific bacteriophage (*Corinebacterium diphtheriae*)
- Speed of reproduction and toxin production could not be in correlation.

Mechanisms of effect of toxin

- **Cytolytic**
  - Toxins reacting with membranes of eukaryotic cells
    - phospholipases C and D
      - Enzymatic hydrolysis
        - » Cleavage of phosphatidylcholine and sfingomyeline
    - toxins binding to a cholesterol in cell membrane
      - So called oxygenlabile hemolysins
        - » in patogenesis of diesease usually low importance only
        - » Exception listeriolysin, O-streptolysin
    - toxin can act as a surface active compound (detergent)
    - toxin entering lipoprotein double layer membrane
      - entering into membrane can produce pores
      - α-toxin *Staphylococcus aureus*
        - » lysis of erythrocytes and other cells

- **Intracellular acting toxins**
  - After binding to specific receptor can enter the cytoplasm
  - Interaction with substrate in cytoplasm
  - Two functional fragments A and B
    - B is bindings to membrane, A is effector
    - First without second inactive (proenzym)
    - After binding to membrane cleavage of disulphidic bridges, B persists linked-up, A enters the cell and triggers effect
    - Similar to lectines

Intracellular acting toxins

Classification:

– **Toxins with transferase activity**

- Transfer of ADP-ribose

1. ADP-ribose + eukaryotic elongation factor 2
   - Arrest of proteosynthesis in cell and cell death
   - Diphteric toxin, A toxin of *Pseudomonas aeruginosa*
2. ADP-ribose + regulatory parts of adenylylcyclase
   - Increased production of cAMP
   - Cholera toxin *Vibrio cholerae*, termolabile enterotoxin of *E. coli*
     - » Increased concentration of cAMP causing secretion of chloride ions and water and stopping absorption of sodium
   - Toxin of *Bordetella pertussis*
     - » Senzibilizing cells for effect of histamine, causes lymphocytoses and lecocytosis, activating Langerhans islets.
   - Increased production of cGMP
     - » termostabile enterotoxin of *E. coli*
3. toxin of *Clostridium botulinum* type C2 a C3, different of botulotoxin
   - transferase toxin of these *C. botulinum* types
     - No proved effect on toxicity
   - iota toxin of *Clostridium perfringens*

Intracellular acting toxins

– Neurotoxins

- **Botulotoxin**
  - Binding to motoric part of neuromuscular plate
  - Presynaptic block of acetylcholine release
- **Tetanospasmin**
  - Toxin actively intaken into neural terminations
  - Retrograde transport to gray do šedé hmoty předních rohů míšních
  - Enter to cells
  - Block of release of inhibition neurotransmitters
- Structure of both toxins similar
  - Endopeptidases depending on zinc
    - » Selective cleaving synaptobrevine (protein present in membrane of synaptic vesicules)
    - » Effect on release of neutransmitters

– Complex toxins

- Anthrax toxin
- Complex toxin composed of three separate proteins
  - **Protective antigen** (PA or factor II)
    - Binding to a specific receptor of eukaryotic cell
    - Formation of secondary receptors for further two proteins
    - Similar function as observed for fragment B of diphteric or cholera toxin
  - **Edemogennic factor** (EF, factor I)
    - Adenylylcyclase dependent on calmoduline
    - Together with protective antigen lowers the activity of neuthrophiles
  - Lethal factor (LF, factor III)
    - The way of effect still not well known
    - Separately inactive.
- All three parts of anthrax toxin trigger the rise of edem and are lethal
- Combination of edemogennic factor with lethal is inactive
- All complex is highly immunogenic, but immunogennic is also separate protective antigen

- **Superantigeny**
  - reagují s buňkami imunitního systému
  - solubilní bakteriální antigeny
    - zejména enterotoxiny a toxin toxického šoku *Staphylococcus aureus*
  - pyrogenní toxiny (erythrogenní) a další toxiny bez označení
    - tvořené *Streptococcus pyogenes*
  - superantigeny mykoplazmat, pseudomonád a enterotoxin *Clostridium perfringens*
  - vedle bakteriálních superantigenů jsou známy obdobné aktivity některých proteinů virových
  - solubilní bakteriální superantigeny
    - aminokyselinové řetězce o molekulové hmotnosti 22-28 kDa
    - mají obdobné uspořádání
    - kódující geny velmi podobné

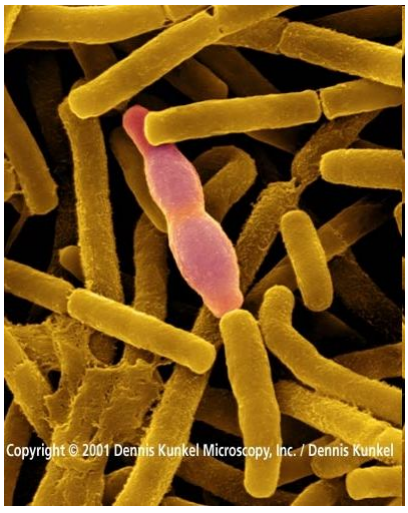
- **Superantigens**
  - Do not require for interaction with immune systém processing with antigen-presenting cells
  - Ability of binding to T lymphocytes receptors
    - β2-domene of MHC II of macrophage
  - After binding trigger the induction of general defensive reaction
    - Polyclonal activation
    - Induction of cytotoxic activity
    - Reaction with receptor for antigen at membrane of lymphocytes
  - Main effect superantigene activity is strong immunomodulation
    - Clinical picture
      - Increased number of cells produced by proliferation initiated by superantigene (elevated number of T lymphocytes with β2 domene) and increased number of CD4, CD8 and B lymphocytes, macrophages, NK cells
      - Release of cytokines, especially TNF, Il1, Il2, Il4, Il10
  - Cooperation of superantigenes on rise of autoimunne diseases
  - Binding of superantigene in area of MHC II at macrophages is displayed in release of solubilble mediators, interleukine 1, TNF, leukotrienes (pyrogennic reaction, loss of weight, somnolence), interleukine 2, γ-interferone
  - Superantigens increase the sensitivity to endotoxins of Gram-negative bacteria
    - Very important during simultaneous infection with bacteria producing superantigen and bacteria releasing endotoxin

Classification of toxins according to organ specifity

- **neurotoxins** (botulotoxins, tetanospasmin),
- **enterotoxins** (choleragen, termolabile and termostabile enterotoxins of *Escherichia coli* and other species of enterobacteria, staphylococal enterotoxins, enterotoxin *C. perfringens*, enterotoxin *Bacillus cereus*)
- **dermonecrotoxins** (diphteria, staphylococal alfa toxin, toxins of *Arcanobacterium haemolyticum, Cor. ulcerans, Cor. pseudotuberculosis*)
- **cytotoxins** (some species of enterobacteria, *Clostridium difficile*)
- **cardiotoxins** (diphteric, oxygenlabile streptolysin)
- **capillarotoxins** (toxins of *Bacillus anthracis, Arc. haemolyticum, Cor. ulcerans, Cor. pseudotuberculosis*)
- **hemolysins** (clostridial, staphylococal, streptococal, tetanolysin, listeriolysin)
- **leucocidins** (*Staphylococcus aureus*)
- **toxins with properties of superantigens** (streptococal pyrogennc toxins, staphylococal toxin of toxic shock)

Toxin of *Bacillus anthracis*

- *Bacillus anthracis*
  - Gram-positive rods
  - *In vivo* in short chains
  - Encapsulation
  - Formation of resistant spores
    - autoclaving



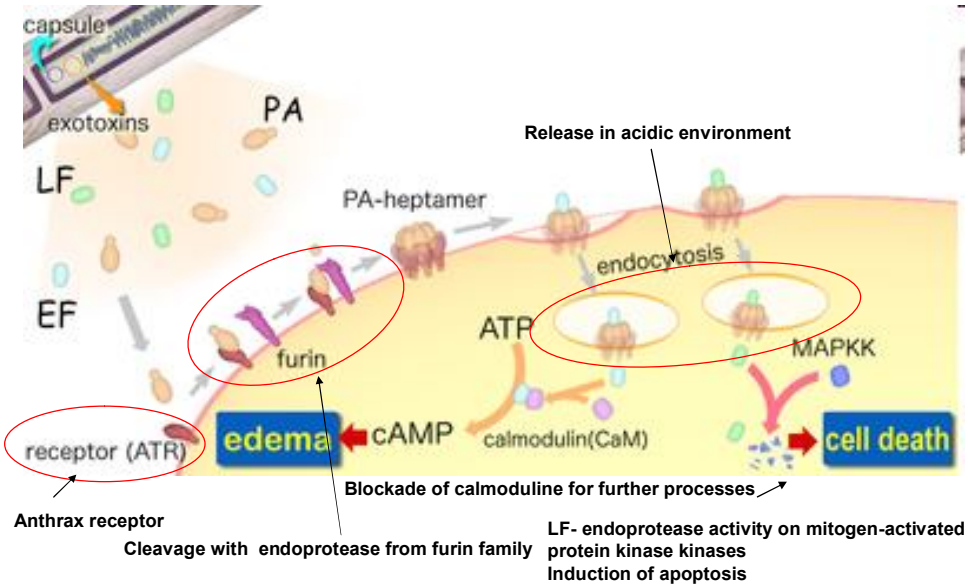
Bacillus anthracis

- **To 30thies worlwide**
  - Today Afrika, middle Asia, south America
  - Pasture × industrial form of anthrax
- **Lower incidence, but possibility of attack**
  - Vaccination, sterilisation of sources, hygiene
  - Ames, Vollum, Sterne
- **Full virulence**
  - Encapsulation + toxin
- **Disease – anthrax – sněť slezinná**
  - After infection **local necroses**
  - **Invasion via lymphatic system into blood circulation**
  - Localisation directly in capillaries
  - **Toxin increases the capillary permeability**
    - „densification" of blood
    - Leakage of liquids into tissue
  - **Septicaemia**
    - **Sudden death becouse of cardiopulmonary failure**
  - Infected perosns spreads the bacillus via extcrets and non-coagulated dark blood leaking all body holes
  - **Skin**
    - Hemorhagic necrosis with pustules and edems, co called pustula maligna - uhlák
  - **Pulmonary**
    - Pneumonia and strong effect on mediastinum
      - Import of bacilli into lymphatic system mediated by macrophages
      - 92% → 45% mortality
  - **Gastrointestinal**

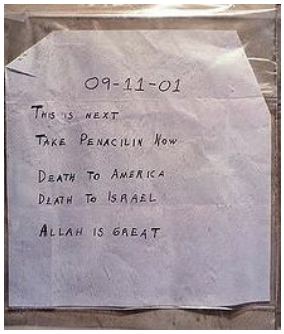
Toxin of *Bacillus anthracis*

- Three components
  - Protective antigen (PA or also factor II)
    - Binding to a specific receptor of eukaryotic cell
    - Formation of secondary receptors for further two proteins
  - Edemogenic factor (EF, factor I)
    - Adenylylcyclase dependent on calmoduline
    - Together with protective antigene lower activity of neuthrophiles
  - Lethal factor (LF, factor III)
- Attack of especially macrophages
  - After internalisation transfer to cytosol
  - Disruption of cellular signal pathways
  - Disruption of cell migration
  - Cell lysis
  - Damage of immune fuction

Effect of anthrax toxin



Gruinard Island



Toxins of *Staphylococcus aureus* types

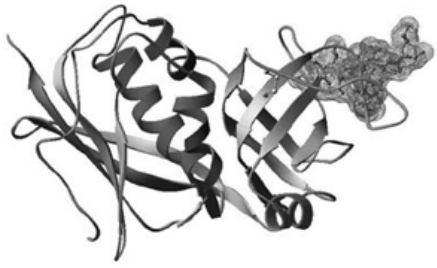
- Enterotoxins
- Exfoliatins (epidermolytic toxins)
- Hemolysin α (alpha toxin)
- Hemolysin β
- Hemolysin γ
- Hemolysin δ
- Hyaluronidase
- Leukocidin (Panten-Valentin toxin)
- Plasmocoagulase (PK)
- Staphylokinase (fibrinolysin)
- Termorezistant nuklease
- Toxin of toxic shock syndrom (TSST-1)

Toxins of *Staphylococcus aureus* types

Enterotoxin B

- 10 antigenic differences A-E G-K
- In Czech mainly A and D

- Protein 28.5 kDa, no sugars and lipids
- Thermostabile
- Pyrogennic toxin
- Alimentary intoxications
  - Mayonnaise, eggs, ice-cream, salads, sweets
- Staphylococcal enterotoxicosis

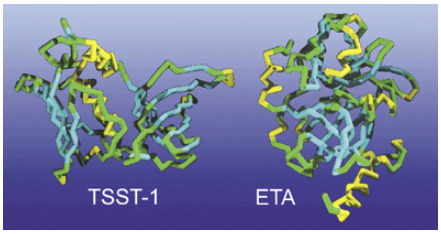


- Source of infection/intoxication
  - Člověk nosič (upto 40 % of population in nosohltanu)
  - Person with purulent disease
- Sites of entry
  - Perorally or inhalation
  - Different symtoms of intoxication
    - Inhalation
      - 3-12 hours
      - Strong fever 39-40°C
      - Tremor
      - Headache and pain of muscles
      - Respiratory distress, non-productive expectoration, sternal pain
    - Peroral entry
      - Interaction with parasympathic ganglia of stomach
        - » Nausea, vomiting, stomach pain, diarrhea
      - Incubation time 1-6 hours
- Complication
  - Hypotension, septic shock, death

- Toxicity
  - ED_{50} 27 μg/kg for monkeys
  - Ten times lower dose can disable human
    - Potential biologic weapon
    - Contamination of water or food
    - Heating kills staphylococcus, but toxin stays resitant
- Mechanism of effect
  - Interaction with immune system
    - Binding to MHC, stimulation of T-lymphocytes proliferation
    - Bacterial superantigen
      - Secretion of cytokines
        - » Interferon, interleukin 1 and 2
- Therapy of disease
  - Supporting
    - Lowering of body temperature
    - Peroral rehydration
    - Supplementaion of electrolytes
- Prevention
  - Hygienic návyky
  - Suppression of risky food

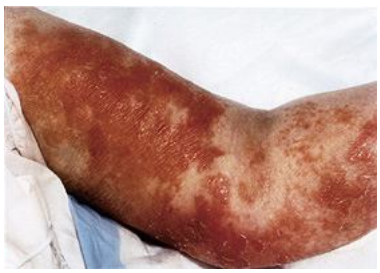
TSST-1

- Antigenic non-uniform protein
- Produced usually by *Staphylococcus aureus* of phage group I or non-characterised tribes
- Synthesis is directed via chromosomal genes
- Super antigen
  - Direct connection with binding molecules of MHC II (ß2 domenes) of macrophages and TCR (Vß domenes) of lymphocytes.
  - Induction of super-production of cytokines
    - Can trigger symptoms of shock syndrome
- TSST-1 possesses mitogennic effects on T-lymphocytes
- Pyrogennic also via direct influence on thermoregulatory centre in hypothalamus
- Inhibits synthesis of macromolecules in endothelias and damages function of many organs
- Reversible hits epithels of kidney tubules in presence of lipopolysaccharide and lowers LPS clearance in liver
  - Enormous sensitivity of organism to effect of LPS of Gram-negative bacteria



Toxic shock syndrome

- Possibility of triggerin during each invasive staphylococcal disease
- Less often during massive colonisation of mucose with tribes producing TSST-1
- Symtoms:
  - Sudden rise of body temperature (>38.9°C)
  - Spotted to diffusive erythema of skin and enanthem of mucose
  - Hypotension, collapses
  - Pannel of symptoms from different organs
    - Diarrhea and vomiting
    - Muscle pain
    - Congestion of mucosa (vagina, esophagus, conjunctivas)
    - Desorientation and consiousness disturbances
    - Liver and kidney function disorders, which can lead to lethal insufficiencies
    - Upto 1-2 weeks from the beginning of disease little desquamation of face and body skin
    - In period of convalescence complete loss of epidermis on palms and feet, fast loss of hair and increased fragility of nails



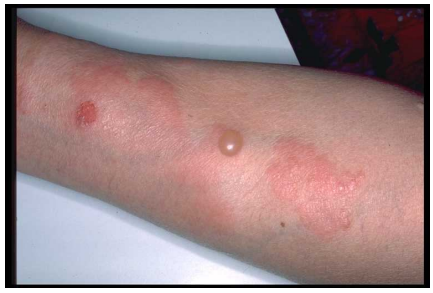
- Exfoliatins (epidermolytic toxins)
  - Low molecular proteins
  - Occurrence in two antigenic typesh A and B.
  - Prevalent is A type
    - Produced by tribes of phage group 11
    - Resistant to boiling for 20 min.
    - In molecule containing Cu
    - Production is directed by chromosomal genes
  - Type B
    - Produced by tribes from other phage groups
    - Is not explicitly thermo-resistant
    - Does not belongs to metalotoxins
    - Production is encoded by plasmide genes
  - Exfoliatins produces so called toxic epidermolysis (exfoliative dermatitis).
  - Sensitivity to this toxin observed only in human and at sucking mouse
  - Although exfoliatins have shown weaker immunogenic properties, type-specific antibodies possess protective character.

Toxic epidermolysis

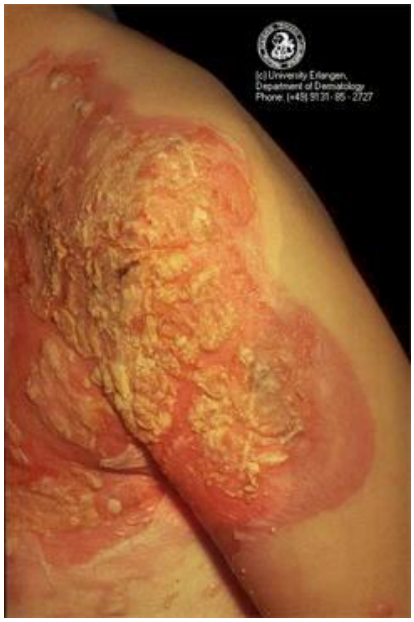
– **Exfoliative dermatitis**

- induced by staphylococcal exfoliative toxin
- into organism released from infected sites
- three forms of disease
  - one localized and two generalized
  - in case of localized form, exfoliatines damage stratum spinosum and stratum granulosum of epidermis. They causes intradermal ruptures with body liquid, ruptures transform to blisters with spontaneous separation of surface layers of skin.

- **Bulos impetigo**
  - pemphigus neonatorum, pemphigoid)
  - localized form
  - newborns and nurse-children
  - on skin separate single blisters with pure liquid
  - their surrounding possesses normal appearance
  - blisters get dry and are healed with crust
  - lesion is without consequences

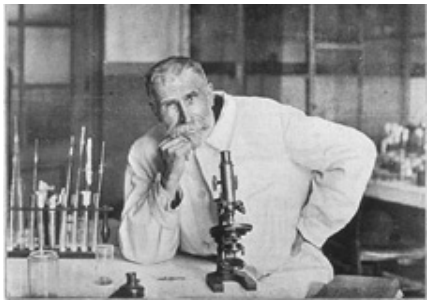


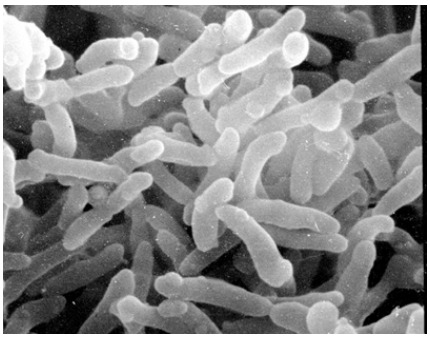
- **Rash types of toxic epidermolysis**
  - Generalized form
  - Characterized
    - Exanthema of body and numbs skin
    - Exanthema of mucosa similar to scarlatine combustion of rush
  - In convalescence occurres also small and laminar desquamation of epidermis
  - Differentiation from scarlatina difficult
    - Missing angina
  - Rash type of toxinosis occurres at patients with insufficient level of antiexfoliatin
  - **SSSS-staphilococcal scalded skin syndrome, Ritter syndrome**
  - Fully developed form of generalized toxinosis
  - Patients completly lacking specific antitoxin in serum
  - Nausea, irritation and sudden rise of body temperature
  - Diffusive erythema
    - Develops perioral
    - After two days expands to whole body
  - Typical is formation of small crusts surrounding mouth and ruptures in corners of mouth and nosolabial furrows
  - On the body surface blisters with pure liquid
  - Surface layer of epidermis is released spontaneously in shreds
  - Similar to scalded skin
  - Exposed places are red, hyperemic, wet, shiny
    - Danger of body liquid leak and superinfection followed by sepsis
  - Exitus is extraordinary, but can occurre during lack of therapy



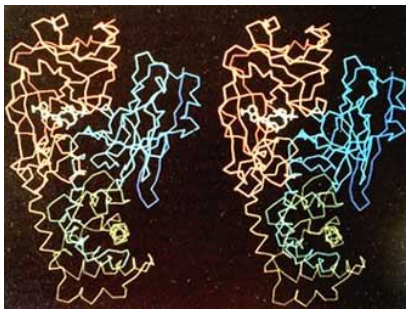
Diphteric toxin

- Product of Gram-positive rod *Corynebacterium diphterie*
  - Club-like or drop-like shape
  - Production of toxin after attack of bakteriophage β
    - Lysogenic conversion
    - Toxigenic and non-toxigenic tribes
      - Disease called diphteria
      - Non-toxigenic tribes only local diseases of respiratory tract or skin, danger for immuno-supressed patients
- Typical exotoxin
  - Protein 60 kDa
  - Released in form of non-active protoxin
  - Proteolytic activation
    - Disulphide cleavage
  - Penetration through membrane similar to tetanospasmin
  - Cytotoxic effect

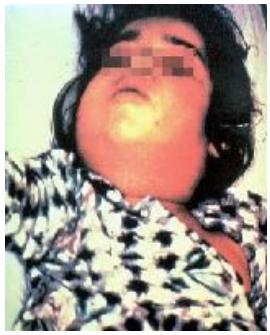


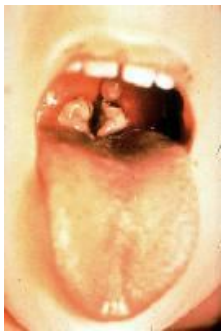


- Diphteric toxin
  - Huge inhibitor of proteosynthesis
  - Lethal dose cca 0.1 μg/kg for human
  - Scale of absorption varies according to site of infection
    - From skin and nose much lower absorption than from esophagus
- Epidemiology of diphteria
  - Infected human or carrier
    - Often asymptomatic carrier
    - Active immunisation
      - Protect against disease
      - Do not protect against carrying
    - Transfer via droplet infection or directly via skin lesion
    - *C. diphterie* resistant to external environmental condition
    - Incubation 2-5 days

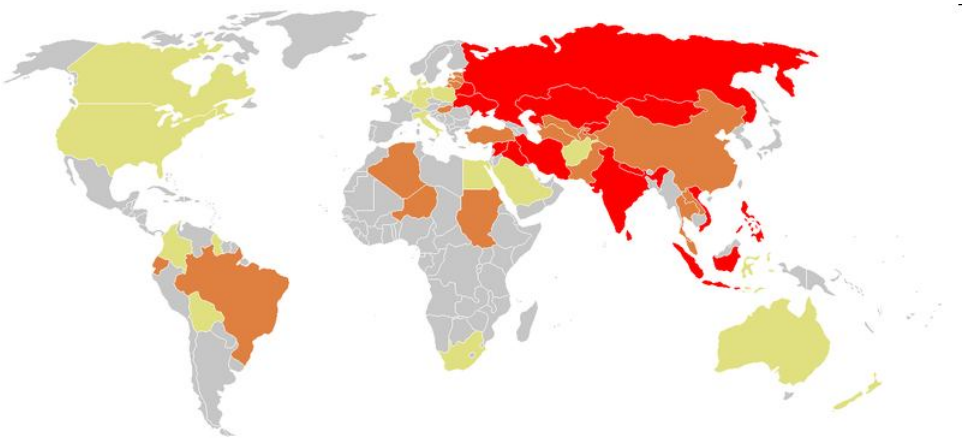


- Clinical picture of disease
  - Diphteric angina
    - Toxin is released
      - Local necrosis
      - Pseudomembranose inflammation
      - Formation of grey pseudomembranes, after removal bloody wounds
    - Malignant diphteria
      - Life endangering state
      - Transfer to palatine
      - High fever
      - Paresis of soft palatine
        - » During drinking liquids leaking through nose
    - Diphteric crup
      - Spread of infection to larynx
      - *Collum caesareum*
      - Suffocation during few hours
  - Invasion of toxin into blood circulation
    - Cardiotoxicity, neurotoxicity, tubular necrosis
  - Vaccinated do not suffer from malignant diphteria or diphteric crup
  - Skin diphteria
    - Inflammatory lesions
    - Homeless people and alcoholics



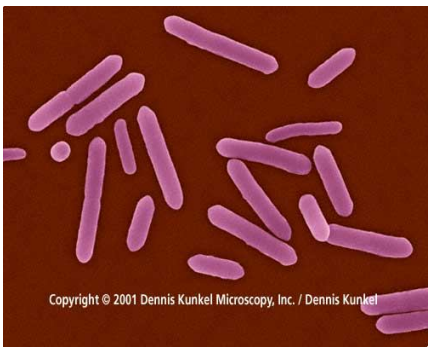


- Expansion
  - Czech Republic - from 1946 vaccination with diphteric toxoid
    - Vanishing of toxigenic tribes
    - Cca 60 % of population in present decrease of antibodies level
      - Possibility of repeated spreading
  - World – especially Russia
- Main therapeutic arrangement
  - Antitoxin
    - Hyperimmune globulin (equine)
    - As soon possible, only free fraction of toxin can be neutralized
  - Antibiotic therapy
  - Vaccination
    - From 9th week of combination Di-Te-Pe
      - 3 doses in one month interval, booster in 6th year of life
    - Travelling to east countries – revaccination

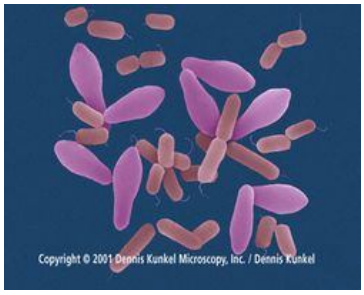


Clostridial toxins

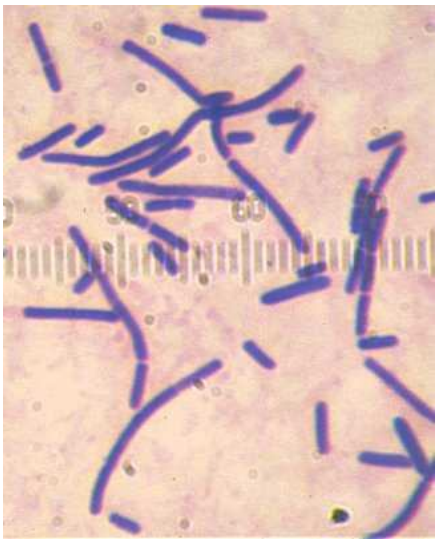
- Toxins of *Clostridium* species
  - 60 Gram-positive anaerobic spore-forming bacteria
  - Most important:
    - *C. perfrigens*
    - *C. septicum*
    - *C. difficile*
    - *C. botulinum*
    - *C. tetani*
  - Toxins of protein character
    - Including tetanospasmin and botulotoxin
    - Dangerous exotoxinemias



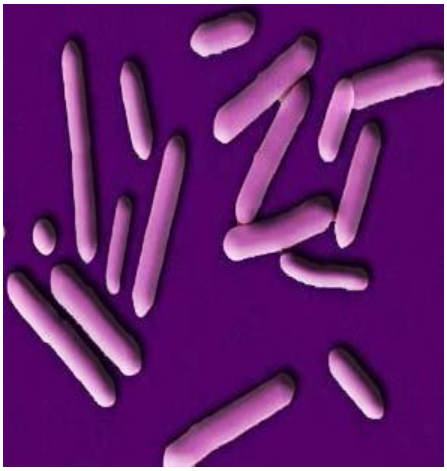
- ***Clostridium perfringens***
  - 5 serotypes A-E
  - **Production of toxins**
    - **α-toxin**
      - Phospholipase C
      - Suppression of contractility of heart muscle
        - » Hypotension, lowered cardiac output
      - Induction of interleukine-8
    - **θ-toxin (perfringolysin O)**
      - Polypeptid
      - Binding to cholesterol of membranes
        - » Only membranes do not containing cholesterol resistant to attack
      - Disruption of permeability, cell lysis
      - Pore-forming cytolysin
      - Presence determine virulence
      - Via endogenous mediators (indirectly) lowers vascular tonus
    - **β-toxin**
      - Tribes C and D
      - Release of catecholamines – hypertension – problem of necrotising enteritis
    - **κ-toxin**
      - Kolagenase
        - » Hydrolysis of colagene of muscles and subcutaneous layer
        - » Facilitation of spread of *C. perfrigens* into tissue



- ***Clostridium septicum***
  - α-toxin
    - Inactive protein
      - Activation by protease
    - Formation of ion channels in cell membrane
    - Binding to glycosylphosphatidylinositol residues in receptors
    - Does not possess phospholipase activity
    - Hemolytic, necrotizing and cytotoxic effects
    - Factor of virulence
  - β-toxin
  - δ-toxin

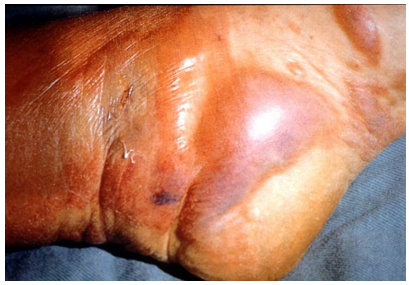


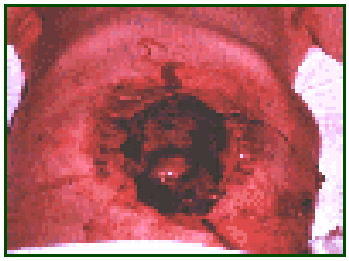
- *Clostridium difficile*
  - Enterotoxin A
  - Cytotoxin B



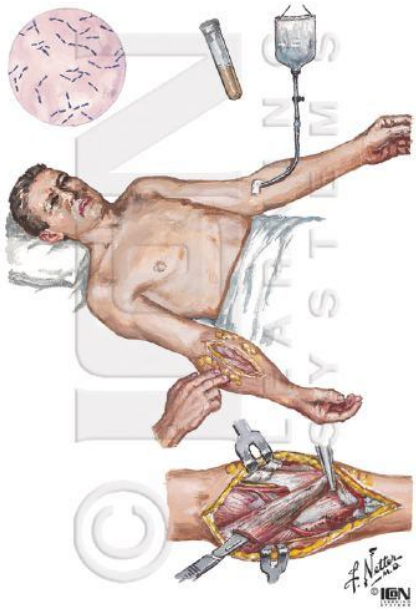
Diseases caused by clostridial infections

- **Gas gangrene**
  - Clostridial myonecrosis
  - In 80 % *C. perfringens*
    - *C. perfirngens* is common component of gut microflora
    - Excrements into soil
      - Spore-formation, long persistence
    - Infection
      - Traumatic or non-traumatic (spontaneous gangrene)
      - Directly into wound via dust or soil
        - » War injuries
      - Contamination of wounds with gut content
      - Gynecologic operations
      - Criminal abortion
    - Necessity of anaerobic conditions, low pH
    - Local symptoms
      - Incubation period dependent on contamination of wound and blood supply from 6 hours to few days
      - Sharp pain in wound
      - Pale skin, then becomes dark to purplish, red-blue blisters, gas in tissues
    - General symptoms
      - Exhaustion, high fever
      - Tachycardia
      - Shock, multiorgane failure





- **Gas gangrene**
  - **Complications**
    - Renal failure
      - Causation
        - » Myoglobine- and hemoglobinuria
        - » Tubul necrosis
        - » Hypotension
  - **High concentration of θ-toxin in site of infection**
    - Tissue damage, killiing of polymorphonuclears and endothelial cells
    - Invasion to further tissues, spreading of zone of destruction
  - **Shock**
    - Caused by α-toxin
      - Cardiotoxicity, hypotension
    - Indirect causation mediated θ-toxin
      - Influence on capillary endothelium, lowering of vascular tonus
  - **Therapy**
    - Succes in case of timely start
    - Surgical removal of infected tissue
    - Penicilline, clindamycine, metronidazol
    - Hyperbaric oxygenotherapy



- **Spontaneous gas gangrene**
  - More often *C. septicum*
    - Growth also under normal oygenation
  - Predisposition
    - Carcinom of colon
    - Surgical performances on colon
    - Hemathologic maligniteis
    - Leukemia, radiotherapy, chemoterapy, weak immunity
- **Necrotising enterocolitis (typhlitis)**
  - Formation of gas in wall og gut, perforation, peritonitis, bacteremia
  - Mainly *C. septicum*
    - α-toxin
  - Without radical therapy (surgery) 100 % of lethality
  - Some forms at long-termed malnutrition
    - Vegetarians
    - Developing countries
    - War
- **Alimentar intoxications**
  - Enterotoxin *C. perfringens* typu A
    - Termolabile
    - Alkaline conditions and presence of trypsine increase
    - Inability of cells of instinal mucasa layer to absorbe glucosis and ions
    - Desquamation of gut mucosal layer
    - Loss of electrolytes
    - Stomach pain and nausea
  - Contaminated food
    - Bad thermal meat treatment, repeated heating of food

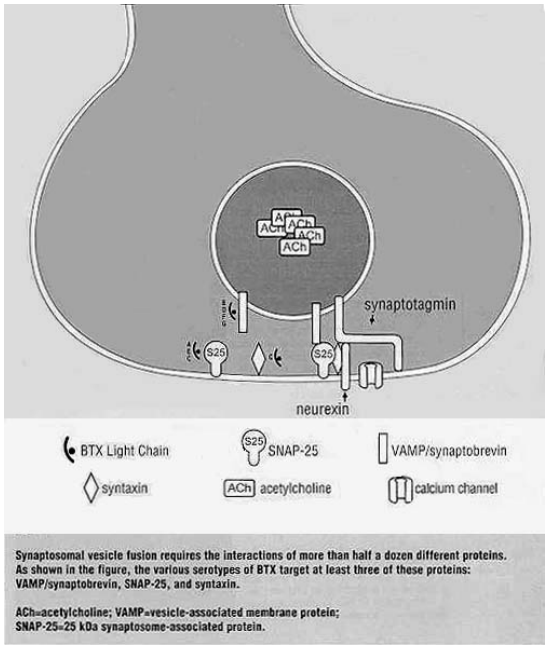
- **Pseudomembranose colitis**
  - Caused by *C. difficile*
  - 3-5 % of population
  - Complication of broad spectral antibiotic therapy
    - Ampiciline, clindamycine, cephalosporine
  - Toxin A (enterotoxin)
    - Binding to colon mucosa layer
  - Toxin B (cytotoxin)
    - Additional damage
  - Inflammatory process
    - Formation of plagues and pseudomembranes on mucosal layer of colon
  - Massive diarrhea, fever
  - Sometimes dehydratation, shock, perforation
  - Nosocomial infections

Botulotoxin

- Group of seven antigenic-different neurotoxins A-G
- Proteins with molecular weight 150 kDa
- Heavy (H) and light (L) chain, disulphidic bridge
- L chain toxic, H chain binding to receptors at presynaptic membrane
- Product of *Clostridium botulinum*
  - Gram positive strictly anaerobic rod
  - Moving
  - Common occurrence in GIT and in dung-fertilized soil
  - Spores resist against several hours of boiling
- Thermolabile toxin
  - 10 minutes of boiling enough for deactivation
- „Sausage poison“ (lat. *botulus* = sausage)
  - Not enough sterilized meat and vegetable canned food
- Intoxication called botulism
  - For human important subtypes A, B, E
  - Classification of intoxication
    - Alimentary
    - New born
    - Early
    - Wound botulism
    - Inhalation botulism
    - Iatrogenic botulism

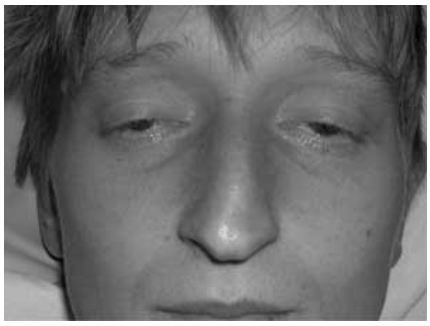
Botulotoxin

- **Mechanism of action**
  - After absorption transport in blood
  - Peripheral neural terminations
    - Binding, inhibition of acetylcholine release from vesucules
  - Serious disruption of peripheral cholinergic transmission
- **Incubation time** 18-36 hours, based on the infection dose
- **First symptoms**
  - Bulbal muscles
    - Mydriasis, diplopia, accomodation disorder, photophobia
- **Progressive development**
  - Vertigo (caused by hypotension)
  - Dryeness in mouth, muscular weakness or paralysis
  - Nausea, vomiting, stomach pain
  - Consiousness stays preserved
  - Death caused by respiratory muscles paralysis



Synaptosomal vesicle fusion requires the interactions of more than half a dozen different proteins. As shown in the figure, the various serotypes of BTX target at least three of these proteins: VAMP/synaptobrevin, SNAP-25, and syntaxin.

ACh=acetylcholine; VAMP=vesicle-associated membrane protein; SNAP-25=25 kDa synaptosome-associated protein.




WALSH COUNTY RECORD

TWELVE DINE WITH DEATH IN GRAFTON FARM HOME

Eleven of Twelve Victims in Poison Food Tragedy

CITY IS SCENE OF MOURNING AS 11 DEAD ARE BURIED

FIVE IN ONE FAMILY ARE VICTIMS OF RARE MALADY

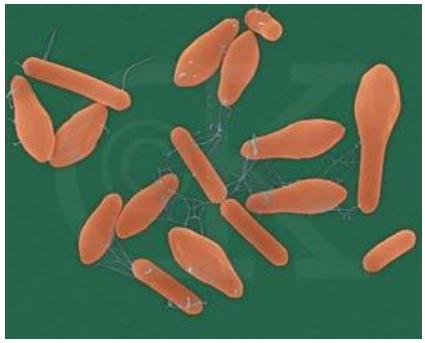
Botulotoxin

- The most redoubtable war poison
  - Toxicity 1 ηg/kg
  - Inhalation of aerosol
    - Similar symptoms as alimentar itoxication
    - Paralysis comes later
  - Asassination of Heydrich
  - War in Gulf
- Usage:
  - Cosmetics
  - Therapy of convulsive neuromuscular diseases
- Therapy of intoxication:
  - Vomiting, gastric lavage
  - Complication is respiratory failure
    - Several weaks of arteficial respiration
    - Guanidin or 3,4-diaminopyridin as support of acetylcholine release
    - Botuline antitoxin
      - Heptavalent equine

Tetanospasmin

- Production organism *Clostridium tetani*
- Two components of tetanotoxin:
  - Neurotoxic component tetanospasmin
    - Disease tetanus
  - Haemolytic component tetanolysin
    - In ethiology of tetanus does not play a role
- Tetanospasmin
  - Polypeptid 150 kDa
  - Two chains
    - Light α-chain
    - Heavy β-chain
    - Disulphidic bridge
  - Penetration to cell
    - Acidic pH - fragment of heavy chain is binding to receptor, formates pores
    - Light chain penetration to cell - neurotoxicity

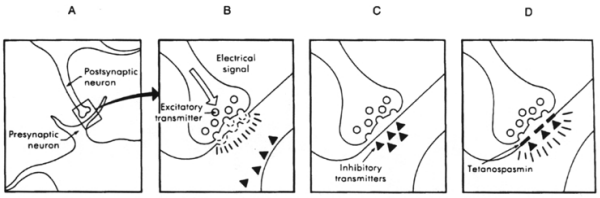
Tetanospasmin



- Highly toxic
  - LD50 for mice 0.002 μg/kg
- Thermolabile
- *C. tetani* sporulates
  - In soil can persist for years
  - For elimination necessary 4 hours of boiling
- *C. tetani*
  - Saprophytic in guts of cattle and other home animals
  - Spores via fertilization into soil
  - Wound contamination
- Disease called tetanus
  - Early infection with serious prognosis
  - After possibility of vaccination on decline in western countries
  - Factor for tetanus manifestation
    - Necrotic tissue, purulent process
    - Presence of strange body inside wound
  - Decreased red ox potential
    - Spores germination
    - Vegetative form
    - Production of toxin

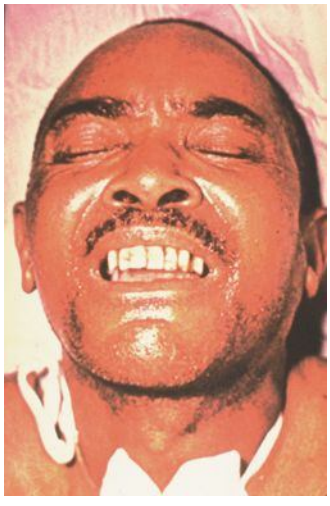
Tetanospasmin

- Transportation of toxin via vegetative nerves to neurons of spinal cord
  - 250 mm per day
- Binding to presynaptic receptors
  - Block of release of glycin and GABA
    - Responsible for inhibitory transmission to aferent motoric neurons
  - Irreversible binding
  - Unlimited muscular contraction
- Affecting also sympathicus
  - Sweating, hypertension to hypotension, arrhythmias
- Incubation period 1-3 weeks
  - Shorter incubation → worse prognosis
    - Dependent on distance of wound from spinal cord and amount of toxin



Tetanospasmin

- 4 clinic forms
  - **Generalized tetanus**
    - Most common
    - Can be triggered from very small wounding
    - Starting
      - Convulsions of chewing muscles (trismus)
      - Increased irritability anxiety, sweating, swallowing problems
    - Progression
      - *Risus sardonicus*
      - Convulsion of dorsal muscles into typical curve
      - Clenched fists
      - Triggering of convulsions by light or touching
      - In full consciousness very painful
    - Terminal stadium
      - Fractures of vertebrae and long bones
      - Laryngospasm, respiratory arrest
      - Lethality ca 50 %
  - **Localized tetanus**
    - Only surrounding of wound, good prognosis
  - **Cephalic tetanus**
    - Head wounds
    - Infection of middle ear
    - Probability of surviving minimal
  - **Tetanus neonatorum**
    - Developing countries
    - Bad hygiene during taking care about umbilical cord
    - 0.5 million of death newborns per year

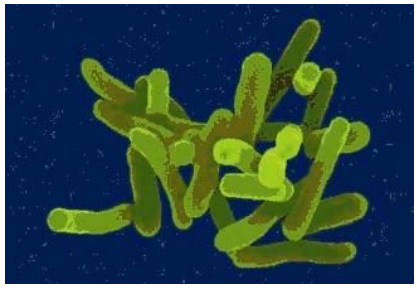


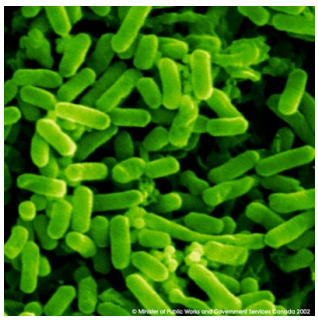
Tetanospasmin

- Therapy
  - Surgical cleaning of wound
    - Does not close
  - Antitoxin
    - Neutralization of toxin before its entry into neuron
  - Myorelaxants
  - Controlled lung ventilation
  - Preventive vaccination
    - 3 doses after months
    - 4th dose in 20th month
    - Re-vaccination in 5th and 14 year
    - Adult each 10 years
    - Booster after wounding

Shigatoxins

- Production organism *Shigella disenteriae*
- Toxic bacterial protein
  - Similar toxicity as botulotoxin 0.002 µg/kg
  - Potential biologic weapon
- Similar toxins produced by *E. coli*
  - Verotoxins
    - Synonyms
      - Verotoxigennic tribes of *E. coli*
      - Shiga-like toxin of *E. coli*
      - Shigatoxin produced by *E. coli*
    - Verotoxin 1 and verotoxin 2
      - Production conditioned by presence of speciphic bacteriophage
- Shigatoxins
  - Proteins coagulatting under heating
  - Sensitive to red ox agents
  - Two chains:
    - A unit
      - Enzymatically active, inhibitor of proteosynthesis
    - B unit
      - Binding to a surface of cell
  - Cytotoxic effects



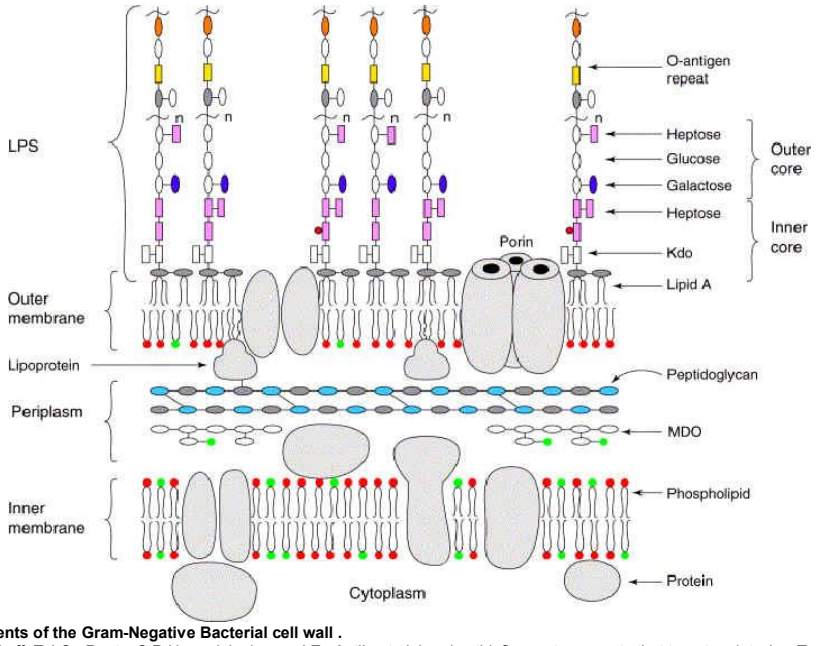


Shigatoxiny

- Initiators of serious diarrheal diseases
  - Potentially lethal
- Cytotoxic effect
  - Endothelium of gut capillaries
  - Kidney glomerules
  - Endothelium of brain veins
- Formed changes – pathologic base for
  - Hemorrhagic colitis
    - Abdominal convulsions, watery diarrhea, blood in faeces
  - Hemolytic-uremic syndrome
    - Complication of *E. coli* infection
    - Diarrhea transferred to bloody diarrhea
    - Uremia, thrombocytopenia, hemolytic anemia, kidney failure
    - Lethality 5 %
    - Possibility of chronic kidney damage
- Infection
  - Reservoir home animals
  - Bacteriophages encoding transfer of genes responsible for toxin production are present in sewage and waste water
  - Alimentary intoxications
    - Badly prepared food (meat) – hamburgers
    - Orofecal transfer possible for children
  - Incubation 2-7 days

Toxic lipopolysaccharides

- **Peptidoglycane layer**
  - Wall of **Gram-negative** and **Gram-positive** stained bacteria
- **Gramnegative bacteria**
  - Peptidoglycane layer
  - Surface layer of outer membrane
    - phospholipids, lipopolysaccharides
    - acidic polysaccharides and proteins (ca 50%)
- Biologically active – **lipopolysaccharide complex**, assigned as **endotoxin**
- Lipopolysaccharide is arranged as double layer, with hydrophilic part composed of polysaccharide, hydrophobic part is lipidic.
- **Structural areas of lipopolysaccharides**
  - **specific polysaccharide (I.)**
    - polymers of several millions of molecular weight
      - polymer composed from oligosaccharides
    - it bears antigenic determinants and determines serologic specifity of bacterial species
  - **Medullar area (II.)**
    - Common for whole group
  - **Lipid A (III.).**
    - medullar oligosaccharide is covalently bonded to lipid A
    - skeleton composed from two molecules of glucosamine, which are connected by phosphate bridges
    - hydroxyl groups are esterified by higher fatty acids
- Single bacterial species are different both in composition of polysaccharide chains and in composition of lipid A
- Polysaccharide part – virulence of bacteria (can contribute to adhesion, activation of complement)
- Lipid A - toxicity



Components of the Gram-Negative Bacterial cell wall .
Wyckoff, T.J.O., Raetz, C.R.H., and Jackman, J.E. Antibacterial and anti-inflammatory agents that target endotoxin. *Trends MicroBio*. 1998. **6**: p. 154-159

- Function of lipopolysaccharide
  - Prevents entering of heavy metals, bile acids or bigger molecules into bacterial cell
- Biologic activity:
  - After release of lipopolysaccharide from outer membrane
    - Release mediated by lysis of bacterial cell
    - In form of free endotoxin
      - Active separation of fragments during the life of bacteria
- Toxicity of endotoxin:
  - During system diseases
  - During bacteremia or presence of bacteria in tissues
  - In lumen of GIT
  - Massive flooding of organism by endotoxin
    - During sepsis induced by Gram-negative bacteria
    - During lysis of bacteria by its own autolytic enzymes (especially at meningococcemias)
    - As a result of cytolysis by complement
    - Via effect of antibiotics

- Endotoxin
  - **participation on the development of toxic shock** in connection with insuficient oxygen supply of various ethiology
    - hypovolemia, stress, operační zátěži apod.
  - Porušená střevní bariéra - umožňuje průnik endotoxinu a jeho uplatnění.
  - Endotoxin stav nemocného zhoršuje
    - možnost letálního konce
  - Za normálních okolností
    - endotoxin z gramnegativních bakterií rezidentní flóry tlustého střeva se vstřebává jen v minimálním množství a stimuluje imunitní systém
  - Endotoxin se uvolňuje do krevního oběhu z různých primárních ložisek gramnegativní flóry
    - nejčastěji při perforaci střeva
    - popáleniny, obstrukce močového traktu
    - infekce žlučníku
  - Nejčastějšími druhy jsou bakterie normální flóry
    - především tlustého střeva (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacteroides fragilis*).
  - Reakce makroorganismu na endotoxin závislá na množství uvolněného endotoxinu
    - nízké dávky
      - interakce s makrofágy, neutrofily, B lymfocyty a komplementovým systémem vyvolává horečku, vasodilatací, zvýšenou syntézu protilátek a zánětlivou reakci
    - vysoké dávky
      - + intravaskulární koagulopatie a šok

- LPS
  - interakce s plasmatickými proteiny a s buněčnými povrchy
  - vazba na plasmatický protein, který se podílí na vazbě LPS na CD14 receptor v buňkách (monocyty, neutrofily).
  - Ovlivňuje i další proteiny
    - Hagemanův faktor
    - Prekallikrein
    - Faktor XI.
    - Kininogen
    - Další faktory zúčastňující se krevního srážení a komplementové kaskády
  - Cílové buňky
    - monocytomakorfágová řada (z nich se uvolňují mediátory)
    - neutrofilní leukocyty
    - B buňky (zvyšuje se tvorba protilátek)
    - endotelie

- **Pyrogenní reakce**
  - vzniká po velmi malých množstvích endotoxinu
  - odpověď na endogenní pyrogeny uvolněné z makrofágů
    - interleukin 1, TNF (tumory nekrotizující faktor)
- **Aktivace komplementového systému** účinkem endotoxinu
  - cytolýza buněk spojená s dalším uvolňováním endotoxinu
  - zvýšení chemotaxe a opsonizace
  - Neutrofily jsou do ložiska přitahovány fragmentem C5a. C3b se chová jako opsonin a tak zvyšuje fagocytózu
  - aktivaci komplementu
    - uvolnění anafylatoxinů (C3a a C5a), které zvyšují kapilární permeabilitu
    - z neutrofilů se uvolňují lysosomální enzymy
    - Aktivace komplementu zprostředkuje zánětlivou reakci

- Interakce endotoxinu s makrofágy
  - nekontrolované uvolňováním cytokinů
    - zejména TNF alfa, interleukin 1 a 6.
  - cytokiny spouštějí uvolňování bioaktivních lipidů a kaskádu dalších cytokinů a kyslíkových radikálů z řady buněk
  - Důsledek
    - zvýšená vaskulární permeabilita, snížení kontraktility srdečního svalu, vasodilatace, plicní hypertenze a disseminovaná intravaskulární koagulopatie.
- IL-1 zvyšuje proliferaci B buněk, které po dozrání produkuji více protilátek, čímž se endotoxin uplatňuje jako nespecifické imunoadjuvans.
  - Popsané účinky menších dávek endotoxinu ukazují, že mohou být pro hostitelský organizmus výhodné (adjuvantní účinky pro tvorbu protilátek, zvýšená aktivita makrofágů, protinádorové účinky).
- Vysoké dávky endotoxinu vedou plynule k příznakům endotoxinového šoku s rizikem letálního konce.
  - zejména vasodilatace a snížení výkonu myokardu
  - poruchy oxidace a mnohoorgánové selhání (S.I.R.S.)
  - spolu s diseminovanou intravaskulární koagulopatií.
  - Klíčovou příčinou hypotenze je uvolnění TNF a interleukinu 1.

- Diseminovaná intravaskulární koagulopatie
  - charakterizovaná vznikem trombů v malých cévách
  - zhoršuje krevní zásobení orgánů.
    - zejména v kůře ledvin, kde vznikají nekrózy.
    - Z dalších orgánů jsou postiženy zejména mozek, plíce (syndrom dechové tísně) a nadledvinky.
  - Endotoxin ovlivňuje srážení krevní 4 způsoby:
    1. aktivuje faktor XII., tzv. Hagemanův, čímž spouští srážecí kaskádu,
    2. ovlivňuje krevní destičky, takže se uvolňuje obsah granul, který se zúčastní na srážení,
    3. iniciuje uvolňování bazických proteinů z neutrofilů a
    4. ovlivňuje endotelie.
  - Poruchy srážení mohou být příčinou vzniku infarktu nadledvin spojeného s náhlou smrtí (Waterhousův a Friderichsenův syndrom u meningokokcemie).