HISTORY OF IMMUNOLOGY

INTRODUCTION:

The immune system is a remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders.

An immune response can be divided into two related activities—

i) Recognition and

ii) Response.

Immune recognition is remarkable for its specificity. The immune system is able to recognize subtle chemical differences that distinguish one foreign pathogen from another. Furthermore, the system is able to discriminate between foreign molecules and the body's own cells and proteins.

Response: Once a foreign organism has been recognized, the immune system recruits a variety of cells and molecules to mount an appropriate response, called an **effector response,** to eliminate or neutralize the organism.

The system is able to convert the initial recognition event into a variety of effector responses, each uniquely suited for eliminating a particular type of pathogen. Later exposure to the same foreign organism induces a **memory response,** characterized by a more rapid and heightened immune reaction that serves to eliminate the pathogen and prevent disease.

Immunology grew out of the observation that individuals who had recovered from certain infectious diseases were thereafter protected from the disease. The Latin term "***immunis"***, meaning **"exempt,"** is the source of the English word immunity, meaning "**the state of protection from infectious disease".**

1) Various reports suggest that the dried crusts derived from smallpox pustules were either inhaled into the nostrils or inserted into small cuts in the skin (a technique called *variolation*). In 1718, **Lady Mary Wortley Montagu,** the wife of the British ambassador to Constantinople, observed the positive effects of variolation on the native population and had the technique performed on her own children.
2) The method was significantly improved by the English physician **Edward Jenner,** in 1798. Intrigued by the fact that milkmaids who had contracted the mild disease cowpox were subsequently immune to smallpox, which is a disfiguring and often fatal disease, Jenner reasoned that introducing fluid from a cowpox pustule into people (i.e., inoculating them) might protect them from smallpox. To test this idea, he inoculated an eight-year-old boy with fluid from a cowpox pustule and later

intentionally infected the child with smallpox. As predicted, the child did not develop smallpox.

3) **Louis Pasteur** had succeeded in growing the bacterium thought to cause fowl cholera in culture and then had shown that chickens injected with the cultured bacterium developed cholera. After returning from a summer vacation, he injected some chickens with an old culture. The chickens became ill, but, to Pasteur's surprise, they recovered. Pasteur then grew a fresh culture of the bacterium with the intention of injecting it into some fresh chickens. Pasteur hypothesized and proved that aging had weakened the virulence of the pathogen and that such an attenuated strain might be administered to protect against the disease. He called this attenuated strain a **vaccine** (from the Latin *vacca*,meaning "cow"), in honor of Jenner's work with cowpox inoculation. Pasteur extended these findings to other diseases, demonstrating that it was possible to **attenuate,** or weaken, a pathogen and administer the attenuated strain as a vaccine.
4) **Pasteur** first vaccinated one group of sheep with heat-attenuated anthrax bacillus (*Bacillus anthracis*); he then challenged the vaccinated sheep and some unvaccinated sheep with a virulent culture of the bacillus. All the vaccinated sheep lived, and all the unvaccinated animals died. These experiments marked the beginnings of the discipline of immunology.
5) 1885, **Pasteur** administered his first vaccine to a human, a young boy who had been bitten repeatedly by a rabid dog. The boy, Joseph Meister, was inoculated with a series of attenuated rabies virus preparations.
6) **Von Behring and Kitasato** demonstrated that **serum** (the liquid, noncellular component of coagulated blood) from animals previously immunized to diphtheria could transfer the immune state to unimmunized animals. the next decade demonstrated an active component from immune serum could neutralize toxins, precipitate toxins, and agglutinate (clump) bacteria. In each case, the active agent was named for the activity it exhibited:antitoxin, precipitin, and agglutinin, respectively.
7) In 1883, even before the discovery that a serum component could transfer immunity, **Elie Metchnikoff** demonstrated that cells also contribute to the immune state of an animal. He observed that certain white blood cells, which he termed **phagocytes,** were able to ingest (phagocytose) microorganisms and other foreign material. Noting that these phagocytic cells were more active in animals that had been immunized, he hypothesized that cells, rather than serum components, were the major effector of immunity.The active phagocytic cells identified by Metchnikoff were likely blood monocytes and neutrophils .
8) Elvin Kabat, a fraction of serum first called gamma-globulin (now **immunoglobulin**) was shown to be responsible for all these activities. The active molecules in the immunoglobulin fraction are called **antibodies.**
9) The 1940s,**Merrill Chase** succeeded in transferring immunity against the tuberculosis organism by transferring white blood cells between guinea pigs. This demonstration helped to rekindle interest in cellular immunity. With the emergence of improved cell culture techniques in the 1950s, the **lymphocyte** was identified as the cell responsible

for both cellular and humoral immunity.

10) Karl Landsteiner, in 1930, for the discovery of human blood groups, a finding that allowed blood transfusions to be carried out safely.

IMMUNOHAEMATOLOGY

Immunohematology is one of the specialized branches of medical science. It deals with the concepts and clinical techniques related to modern transfusion therapy. Efforts to save human lives by transfusing blood have been recorded for several centuries. The era of blood transfusion, however, really began when William Harvey described the circulation of blood in 1616.

Blood transfusions continued to produce unpredictable results, until Karl Landsteiner discovered the ABO blood groups in 1900, which introduced the immunological era of blood transfusion. A year later the fourth blood group was discovered, but it was not until 1937 that the Rh factor was discovered.

Landsteiner discovered that the blood antigens (ABO) present on RBCs (RBCs) would react with their respective antibodies present in plasma, and that this reaction had great clinical significance.

It became clear that the incompatibility of many transfusions was caused by the presence of certain factors on red cells now known as antigens.

Two main postulates were also drawn by this scientific approach:

1. Each species of animal or human has certain factor on the red cell that is unique to that species, and
2. Even each species has some common and some uncommon factor to each other. This landmark event initiated the era of scientific – based transfusion therapy and was the foundation of immunohematology as a science.

Many more blood group antigens have been identified subsequently, mostly by studying antibodies in patients who had received multiple blood transfusion or mothers of infants with hemolytic disease. The main blood group systems with the dates of their discovery are shown below.

ABO	1900	Duffy	1950
MN	1926	Kidd	1951
P	1926	Diego	1955
Rh	1940	Yt	1956
Lutheran	1945	Kg	1962
Lewis	1946	Dombrock	1965
Kell	1946	Colton	1967

Some antigens have been identified that occur very rarely, being limited to certain individuals or families. These have been termed ‘private antigens’.

Immunohematology is the study of blood group antigens and antibodies and their interactions in health and disease. Both the cellular elements and the serum constituents of the blood have distinct profiles of antigens. There are multiple systems of blood cell groups, all of which

may stimulate antibodies and interact with them. These may be associated with erythrocytes, leukocytes, or platelets.

Blood Groups

ABO antigens and antibodies

The ABO blood group is the most important of all the blood group systems. There are four different ABO blood groups (see Table1), determined by whether or not an individual's red cells carry the A antigen, the B antigen, both A and B antigens or neither.

Normal healthy individuals, from early in childhood, make red cell antibodies against A or B antigens that are not expressed on their own cells. These naturally occurring antibodies are mainly IgM immunoglobulins. They attack and rapidly destroy red cells carrying the corresponding antigen. . For example, anti-A attacks red cells of Group A or AB. Anti-B attacks red cells of Group B or AB.

Name of Blood Group	Antigens present on the red cell surface	ABO antibodies present in the plasma
Type O	nil	anti-A and anti-B
Type A	A antigen	anti-B
Type B	B antigen	anti-A
Type AB	A and B antigens	nil

If ABO incompatible red cells are transfused, red cell haemolysis can occur. For example if group A red cells are infused into a recipient who is group O, the recipient's anti-A antibodies bind to the transfused cells. An ABO incompatible transfusion reaction may result in overwhelming haemostatic and complement activation, resulting in shock, renal failure & death.

Rhesus D (RhD) antigen

There are more than 40 different kinds of Rh antigens. The most significant Rh antigen is RhD. When RhD is present on the red cell surface, the red cells are called RhD positive. Approximately 80% of the Australian population are RhD positive. The remaining 20% of the population that lack the RhD antigen are called RhD negative

Antibodies to RhD develop only after an individual is exposed to RhD antigens via transfusion, pregnancy or organ transplantation. Anti RhD (or anti-D) antibodies destroy RhD positive red cells and can lead to haemolytic transfusion reactions. This is of particular importance in pregnancy where anti-D antibodies can cross the placenta from mother to unborn child and lead to haemolytic disease of the newborn.

a general rule, RhD negative individuals should not be transfused with RhD positive red cells, especially RhD negative girls and women of childbearing age. If transfusion of an

RhD positive product to RhD negative recipient is unavoidable a haematologist should be consulted and administration of anti-D immunoglobulin considered.

When a transfusion is given, it is preferable for patients to receive blood and plasma of the same ABO and RhD group. However if the required blood type is unavailable, a patient may be given a product of an alternative but compatible group as shown below

Blood Compatibility		
Patient Type	**Compatible Red Cell Types**	**Compatible Plasma Types (FFP & Cryoprecipitate)**
A	A, O	A, AB
B	B, O	B, AB
O	O	O, A, B, AB
AB	AB, A, B, O	AB
RhD Positive	RhD Positive RhD Negative	RhD Positive RhD Negative
RhD Negative	RhD Negative	RhD Positive RhD Negative

Group O RhD negative (O negative) red cells have neither ABO nor RhD antigens on their surface. O RhD negative red cells are issued in emergency situations where life saving transfusion is required prior to completion of a cross match. Both RCH and RWH blood banks maintain a reserve of 5 emergency O RhD Negative red cells. (Click here for further information on emergency blood release). Group O is often referred to as the universal red cell donor.

Group AB individuals have neither anti-A nor anti-B antibodies in their plasma. Group AB plasma can therefore be given to patients of any ABO blood group and is often referred to as the universal plasma donor.

Avoiding ABO incompatible transfusions

Most ABO incompatible transfusions occur as a result of improper patient identification at the time of collection of the pre-transfusion sample or administration of the blood product. The pre-transfusion check is carried out at the bedside by 2 members of clinical staff to ensure the right blood is transfused to the right patient. Positive patient identification prior to blood sample collection and labelling the specimen tube at the bedside is critical for accurate sample collection.

Other blood cell antigen-antibody systems

There are many other antigen systems expressed on red cells, white cells and platelets. Transfusion can cause antibodies to develop in the recipient. Some of these antibodies can cause transfusion reactions or damage the foetus. The purpose of pretransfusion testing (or cross matching) is to detect potentially harmful antibodies in a patient before transfusion and where possible select red cell units that will not react with them.

HOST PARASITE RELATIONSHIPS

INTRODUCTION:

a) Healthy individuals are **INFECTED** and are being infected anew constantly.

b) Some of these organisms maybe **PATHOGENS** (more frequently among the transient flora group).

Some among the normal flora may be **OPPORTUNISTS**.

c) Our relationship with microbes is very dynamic:

THERE IS A BALANCE BETWEEN:

The disease causing properties of the microbes and the antimicrobial defenses of the host.

d) **INFECTIOUS DISEASE** is disease caused by pathogenic microorganisms.

Pathogenic = disease causing agent

Virulence= degree or intensity of pathogenicity

Disease =abnormal state, deviation from a state of wellness or health

Contamination means that microorganisms are present.

Infection, when parasitic microorganisms increase in number either within or on the body of the host.

e) Whether or not you will catch a disease depends on:

(1) YOU: your health, nutrition, immune status.

(2) The pathogen's **VIRULENCE**

(A) How **TOXIC** the organism is

(B) How **INVASIVE** the organism is

IV. SYMBIOSIS, COMMENSALISM, MUTUALISM, PARASITISM

- ✓ **Mutualism** is a symbiosis in which both members benefit from the relationship.
- ✓ **Parasitisms** are those relationships in which one member benefits, and the other one is harmed in some way.
- ✓ **Commensalism** is a relationship in which one member benefits, and the other one neither benefits nor is harmed.
- ✓ Imposed on the idea of **opportunism**

OPPORTUNISTS - These are organisms that normally don't cause disease but will if given an opportunity: As in secondary infections.

-If resistance is low: - ***Pneumocystis carinii*** - pneumonia in immunocompromised individuals. ***Neisseria miningitidis*** - meningitis in children, 5-15% carriage rate in apparently healthy people.

- If they get into the wrong place. Ex: ***E. coli*** - Normally in the intestine, but if in the bladder or peritoneal cavity - Big problem!. Susch as when one sees peritonitis after a ruptured appendix.

Streptococcus pneumoniae - Pneumonia after influenza or other respiratory tract infection particularly in the elderly people.

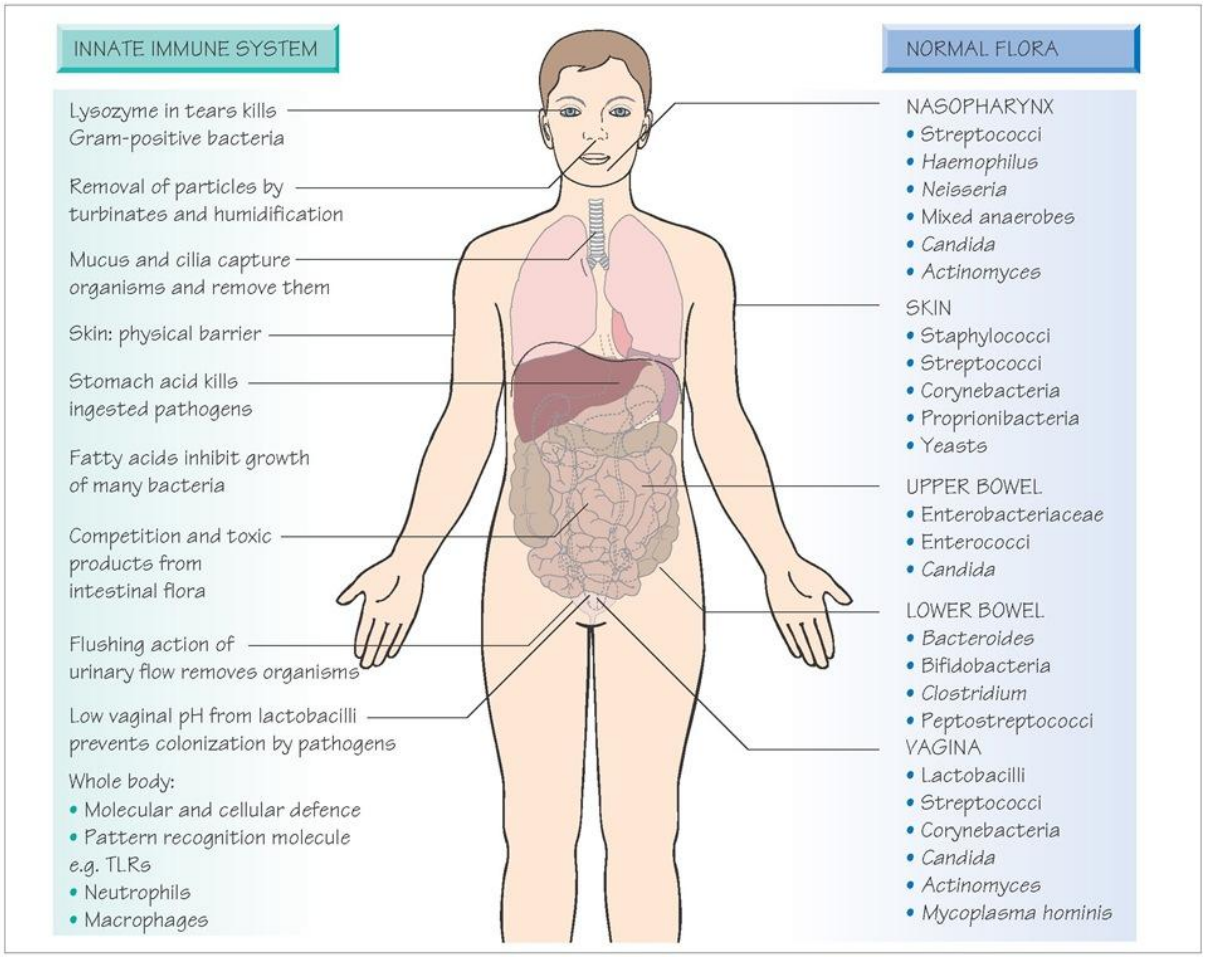
Candida albicans – Vaginitis and thrush

Pseudomonas aeruginosa - Infections of burns and infections of the lungs in cystic fibrosis

Staphylococcus aureus - Skin infection and toxic shock syndrome

II. THE NORMAL FLORA

* The human adult is estimated to have 10^{13} human cells and 10^{14} bacterial cells.



* In all honesty, we are a cylinder with a hole through the center. Just about every surface is colonized.

There are areas:

- **THE STOMACH** - was thought to be free of organisms -- but recently shown that some organisms can survive --- ***Helicobacter pylori***---- This is probably not considered normal flora since it is now known to cause ulcers.
- **THE BLADDER** and the **LOWER REACHES OF THE RESPIRATORY TRACT**. These areas can be transiently infected but normal clearance mechanisms exist to get rid of the intruders.
- We are colonized at birth with ***Lactobacillus*.**
- Over time we acquire and establish populations of: **COLIFORMS** - (*Escherichia coli* and other enteric gram negative rods) - **intestines**
- *Staphylococcus aureus, Staphylococcus epidermidis, Proprionibacterium acnes* and other diptheroids- **skin**
- *Streptococci* (*viridans* and *pneumoniae*) as well as some anaerobes - **mouth**
- *Lactobacilli* - vagina

III. **BENEFICIAL EFFECTS OF THE NORMAL FLORA**

1) Antagonism - Competition and the production of inhibitors (bacteriocins), If normal flora is destroyed ex.: Pseudomembrane colitis – *Clostridium difficile* and *Candida* infections - Thrush, vaginitis caused by antibiotics, douches and deodorants;

2) Production of nutrients- Vitamins B and K in the intestines by *E. coli*

3) Stimulate maturation of the immune system

HOW TO DETERMINE IF AN ORGANISM IS THE **ETIOLOGIC AGENT OF DISEASE**

KOCH'S POSTULATES:

1. The agent must be observed in every case of the disease.
2. The agent must be isolated from a diseased host and grown in pure culture.
3. When purified agent is inoculated into a healthy host, it must cause the same disease.
4. The agent must be reisolated from the newly infected, diseased host, and be identical to the previously identified causative agent.

CLASSIFICATION OF INFECTIOUS DISEASES.

How they behave within a population?

I. Spread within a population

1) COMMUNICABLE DISEASES = CONTAGIOUS, easily spread from host to another either directly or indirectly, ex.: influenza, common cold, tuberculosis, chicken pox, measles, genital Herpes.

2) NON-COMMUNICABLE DISEASES a disease that is not spread from one host to another. ex.: tetanus, food poisoning, subacute bacterial endocarditis, etc.

II) Frequency in a population:

a) **Epidemiology**: study of factors and mechanisms governing spread of disease within a population, incidence, spread, control, prevention. (what, how, when).

Diseases in a population:

a) **Sporadic disease**: outbreak occurs in population occasionally and at irregular intervals eg, many gastrointestinal diseases from contaminated food. (Typhoid fever)
b) **Endemic disease**: constantly present at low level eg, colds
c) **Epidemic disease**: outbreak occurs in unusually high numbers in a population eg, mumps in elementary schools, influenza.
d) **Pandemic disease**: epidemic over a large geographical area (such as the world). eg, worldwide influenza outbreak in 60s, AIDS and coronavirus.

SEVERETY OR DURATION OF DISEASE:

a) Duration:

1) **Acute disease** – develops rapidly but ends shortly (influenza, herpes)
2) **Chronic disease** – slow development but continuous for long period of time (TB, infectious mononucleosis [EBV], hepatitis B
3) Latent disease – agent remains inactive then reactivates (TB, toxoplasmosis, shingles [VZV])

b) Severity:

1) Local VS systemic – infectious agent remain in one area in the body (abscesses in teeth, skin
infections) or they can be spread through the body by the blood (measles, chicken pox).
2) Primary vs secondary – acute infection that causes initial illness or caused by an opportunistic
pathogen after body weakened by initial infection.
3) Bacteremia vs septicemia – bacteria are present in the blood. When bacteria are actually growing and dividing in the blood.

SPREAD OF INFECTION IN A POPULATION

* Need to know the reservoir and mode of trasnmission

a) RESERVOIR - Can think of the reservoir as the source of the organism

1 Human source:

Sick human -- Acute illness (rapidly developing and often rapid resolution - get better or die. Ex: influenza, chicken pox, cholera, strep throat.

Carrier human -- Inapparent infections, subclinical infection, chronic infection. Ex: tuberculosis, Epstein-Barr infection (mononucleosis), syphilis, HIV infection, Hepatitis B

2) Animal source: wild animal? domestic?

Zoonosis -- primarily an animal disease and then spread to humans. Examples of diseases with a significant animal reservoir: rabies, plague, leptospirosis, lyme disease, Toxoplasmosis, psittacosis, salmonella food poisoning.

3) Non-living source:

- a) **Soil** harbors organisms that cause a variety of infectious diseases. Ex: tetanus, botulism, anthrax, *Pseudomonas* infections, a variety of fungi.

- **Water** is often contaminated and can serve as a vehicle of infection. Ex. giardiasis, typhoid fever, amebiasis, leptospirosis -- note that water is easily contaminated by human and animal feces.

MODE OF TRANSMISSION

1. **CONTACT TRANSMISSION:**

a) Direct contact -- Touch, kiss, sex, animals

b) Indirect: - **Fomites** (Inanimate objects that serve as means of spread) -- doorknobs, towels,

phones, needles and other medical equipment.

c) **Droplet transmission:** droplet nuclei - travel short distances - Important in many respiratory infections

2. **VEHICLE TRANSMISSION:**

a) Waterborne- **oral-fecal transmission**

b) Foodborne—unproperly cooked, fecal contamination

c) Airborne -- droplet nuclei in dust -- must travel more than 1 meter.

3. **Vector** -- and infected animal (usually an insect) that transmits to humans

Biological transmission -- The animal host is needed by the microbe for some metabolic or other essential process.

Ex.:malaria protozoan- mosquito; Lyme disease spirochete -- tick; sleeping sickness protozoan -- tsetse fly; the plague bacterium -- flea and rodent (rat)

Mechanical transmission -- the animal host host picks up the microbes and moves them around. Ex.: flies landing on feces or dead animals. cockroaches and lizards rats can do the same thing.

THE VIRULENCE FACTORS OF MICROORGANISMS

a) PATHOGENICITY vs. VIRULENCE

- **Pathogenicity** - The ability of an organism to cause a disease. A taxonomically significant attribute generally ascribed to a species.

Generally organisms referred to as pathogens have a high probability of causing an infection (and more reasonably should be called frank pathogens!), but under unusual

circumstances any microorganism, even a non-pathogen, might be capable of causing an infection.

Virulence - The degree of pathogenicity. An attribute generally ascribed to a strain.

* This involves **INVASIVE FACTORS** and **TOXICITY FACTORS**

* Organisms have to get in first (How?)—

The portals of entry:

- Mucous membranes - mouth, nose, eyes, respiratory tract, GI, GU.
- Breaks in the skin
- Breaks into the tissues below the skin into the tissues, veins, or arteries -- PARENTERAL

Development of disease:

2) ORGANISMS HAVE TO ADHERE -- THEY HAVE **ADHESINS**

CAPSULE—

Helps to attach but also escape from phagocytosis. ex.: *Streptococcus pneumoniae, Klebsiell a pneumoniae, Streptococcus mutans* – dextran and tooth decay.

FIMBRIAE (**PILI**) -- Are Often Tissue Specific. ex.: *E. coli* strain differences in tissue specificity due to different types of fimbriae - urinary tract strains differ from enteropathogenic strains in the type of pili they make. Whooping cough bacterium – *Bordetella pertusis* attaches specifically to respiratory epithelium cilia.

Neisseria gonorrhea - has pili and outer membrane proteins that allow it to stick to urethral and vaginal epithelium, non-ciliated fallopian tube cells, sperm cells and neutrophils; *Streptococcus pyogenes* - protein F attaches to pharyngeal epithelium, protein M attaches to keratinocytes in the skin both proteins are are part of the fimbriae of these organisms.

THE **CELL WALLS** of some bacteria have virulence promoting properties. ex.: *Streptococ cus pyogenes* – protein G binds to the back end of antibodies preventing their normal function - this is a CLOAKING DEVICE for the bacterial cell and it helps the cell avoid phagocytosis. *Staphylococcus aureus* has protein A - which works the same way.

Mycobacterium tuberculosis has wax D and sulfolipids which inhibit the killing mechanisms of phagocytes.

The outer membrane of Gram negative organisms **(endotoxin, LPS, contains lipid A).** The structure is shed periodically but in large scale when the organism dies. Profound effect on humans: fever, weakness, aches, schock, hemorrhage,etc. These effects are caused by the release of interleukin (IL)-1 and tumor necrosis factor (TNF) by macrophages.

BACTERIAL ENZYMES ASSOCIATED WITH VIRULENCE - many are exoenxymes or secreted enzymes.

- Leukocidins -- destroy phagocytic leukocytes, which then release their own digestive enzymes onto tissues. *S. aureus* and *S. pyogenes*.
- Hemolysins -- destroy RBC'S -- *S. aureus*, *S. pyogenes* and *C. perfringes*
- Coagulase -- makes a fibrin clot around the organism. -- *S. aureus*
- Bacteria kinases -- digest fibrin clots -- streptokinase and staphylokinase. can be used therapeutically to dissolve blood clots.
- Hyaluronidase -- dissolves hyaluronic acid
- Collagenase (gelatinase) -- dissolves collagen -- *C. perfringes*
- Siderophores -- scavenge iron

a) BACTERIAL TOXINS -- EXOTOXINS (many are enzymes)

- Can be classified as **CYTOTOXINS, NEUROTOXINS, ENTEROTOXINS.**

Diptheria toxin -- phage with TOX gene. Toxin inhibits protein synthesis in eukaryotic cells

- **Erythrogenic toxin** -- This is a **SUPERANTIGEN** -- host response causes fever and rash and damage to capillaries.
- **Botulinum toxin** -- hits the neuromuscular junction - No acetylcholine release - flaccid paralysis.
- **Tetanus toxin** (tetanospasm) -- hits the central nervous system - Inhibits the firing of the inhibitory motor neurons - Spastic paralysis.
- **Cholera toxin** (choleragen) -- Stimulates adenyl cyclase in the enterocytes - which then dump water and electrolytes into the small intestine.
- ***E. coli* enterotoxin** -- very similar to cholera toxin
- ***S. aureus* enterotoxin** -- has cholera like activity but is also a superantigen -- Activates T-cells to release a lot of interleukin-2 **(IL-2)** and Tumor necrosis factor (**TNF).**

Some bacteria have enzymes and properties that allow them to survive inside phagocytic cells. These include *Mycobacterium tuberculosis*, *Salmonella typhi*, and *Neisseria gonorrhea*.

b) The outer membrane of Gram negative organisms (endotoxin, LPS, contains lipid A).

This structure is sloughed off during the life of the organism and is shed in large scale when the organism dies. Has profound toxic effects on the human. can result in fever, weakness, aches, shock, hemorrhage, miscarriage, disseminated intravascular coagulation. These effects are caused by the release of interleukin-1 (IL-1) and tumor necrosis factor (TNF)-alpha by macrophages in an apparent over-reaction. These are cytokines which affect many aspects of the inflammatory and immune responses.

The landmark of endotoxemia are: 1) Fever and 2) Toxic shock.