Barriers to Infection

Normal Microbiota
what, where, when, why & how?
What we know & don't know
What is the role of? Importance of?
As opportunistic infections?

Innate Immunity

External Barriers to Infection

Constantly exposed to microbes but don't develop diseases
Resistance to disease is due to:
(1) External barriers physical & chemical
(2) Complex systemic defence systems - innate - adaptive

(1) (1) & (2) Communicate with each other
to protect against invasion by pathogens

First barriers to cross for any infectious agent to the normally sterile areas of the body are:
The skin
Conjunctivae of the eye
Mucous membranes - respiratory tract - alimentary tract - urogenital tract
Physical Epithelial Barriers

- Epithelial cells joined by tight junctions
- Exfoliation of surface cells
- Mucous flow by ciliated epithelia (respiratory tract)

Defences of the Skin

Skin is important barrier to pathogens
Surface layer - epidermis - consists of dead cells
Generally surface is dry & acidic; not microbe friendly
Viruses cannot replicate in dead cells
Dead cells of skin, constantly sloughing off plus anything attached to it, e.g. Microbes
see fig 12.2 from recommended textbook

Epithelium - Skin

Skin barriers to infection

As the cells of the dermis grow out into epidermis produce high levels of keratin not utilized readily by microbes
Dead skin cells not being nutrient rich - microbes not supported
Some microbes do manage to survive on skin as part of the normal microbiota
These microbes tend to play protective role by competing for colonization sites and nutrients

Breaches in the skin

Bites, cuts, burns, trauma allow surface or environmental bacteria into the tissue... cause infection

Burns destroy specific and non-specific defences by destroying the tissue......? Survive 2nd infections?

Mucosal membranes as barriers to infection

Although internal surfaces, intestinal & respiratory tracts, vagina and bladder are all constantly exposed to material from external environment
Lining of GIT, lung airways & bladder consist of single layer of cells (Structurally thin to allow secretions, passage of gases)
Single layer of cells = Mucosal epithelial cells
Fragile barriers are protected by thick, sticky layer of mucin (mucous) Mucin= proteins + polysaccharides
Role is to trap microbes
Prevent microorganisms reaching epithelial cells
In vagina & intestinal tracts, mucous also lubricates against mechanical damage to the epithelial cells
Mucous membranes as barrier to infection

- Mucin produces antimicrobial substances
- Lactoferrin - iron binding protein, deprives organism of iron
- Lysozyme - enzyme that digests cell wall of bacteria
- Defensins - small protein that form holes in microbial membranes
- Mucin is constantly being shed and replaced so trapped microbes constantly expelled from the body
- Epithelial cells also replaced frequently, so any attached microbes that get through mucin will be shed.
- Phagocytes in MALT and GALT will engulf and destroy invaders

See figure 12.3 in Microbiology, Diversity, Disease and Environment textbook for diagram of sloughing of mucin layer

Respiratory Tract: mucociliary escalator

- Respiratory tract constantly exposed to particulate matter and droplets
- Nasal hairs favour trapping of particles by mucous membranes
- Nasal turbinates present large surface for trapping inhaled particulate matter
- Trapped particles are transported by ciliated epithelium to oropharynx
- These secretions are periodically swallowed
- Small particles can pass into the lower RT where mucociliary escalator directs the flow of secretions up to oropharynx
- Smallest particles <5μM are ingested by alveolar macrophages
- Normal flora also protects against colonization

Gastrointestinal Tract

- Constant contact with organisms via food and water
- Intricate defense systems include:
  - Mucus
  - Gastric acid
  - Pancreatic Fluids
  - Bile salts
  - IgA
- Risk occurs when:
  - Exposure to virulent organism
  - Decrease in gastric acid production
  - Antibiotic therapy
  - Abnormal GI motility

Respiratory Tract

- Constant exposure to thousands of potential pathogens
- Unique defence structure:
  - Mucociliary escalator
    - Particles >5 micron: cleared by mucociliary escalator
    - Particles <5 micron: cleared by macrophages & PMNs
- Risk occurs when:
  - Mucociliary system is damaged (smoking, COPD, pathogens)
  - Exposure to organisms which adhere to respiratory epithelium
  - Patient is immunocompromised
**Alimentary Tract – barrier to infection**

Constant swallowing acts to flush microbes into stomach

Normally acidic stomach eliminates majority of ingested microbes

- Achlorhydria (low acid) resulting from disease / ulcer drugs
  - Higher association with enteric infections
  - Require a lower inoculum of Salmonella typhi than healthy individuals

Peristaltic activity of colon... flushes out microbes

Augmented peristalsis as in diarrhoea induced by enterics serves to flush out unwanted microbes

**The Eyes**

Protective barrier is flushing by tears

Tears have lysozyme - lyses cell walls of microbes

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**Chemical Epithelial Barriers**

- **Enzymes**
  - Lysozyme (tears, saliva, sweat)
  - Pepsin (stomach)

- **Acid/Base**
  - Fatty Acids/amino acid (skin)
  - Gastric acids (stomach)

- **Antimicrobial**
  - Transferrin (mucus), Defensins

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**Terms and definitions**

Normal microbiota: microbes that colonize various parts of the body and exist symbiotically (live together) for life

- **Resident:** "long term locals" usually found at a particular site
- **Transient:** "visitors" found at a site transiently

- **Mutualists:** provide beneficial effects such as producing acid/ lowering pH & blocking colonization by more dangerous pathogens
  - E. coli synthesizes vitamin K and some B vitamins that are absorbed into the bloodstream for use by the host. The large intestine provides nutrients to the E. coli.

- **Commensals:** most normal microbiota are commensals
  - they neither harm nor help the host

- **Opportunists:** usually commensals or mutualists, but have the ability to become parasitic & harm the host
  - E. coli is usually a mutualistic organism, but if it finds its way to the urinary bladder it may cause urinary tract infections.
Normal microbiota = normal microflora

Born “Germ free” ... acquire first microflora in the first hours to days after birth
Spectrum of microbes changes with growth & development of the person
In cell numbers, bacterial > mammalian!
comprising 10
14 microbes:10
13 mammalian cells
External surfaces: skin and conjunctiva of the eye
Internal surfaces: linings of the digestive, respiratory & urogenital tracts
Internal structures and organs are usually sterile
eg. Bone, heart, liver, kidneys, uterus, spinal cord and brain
Normal microbiota may be harmless, beneficial or disease causing

Beneficial aspects of normal microbiota

- bind to specific sites on host cells effectively blocking the sites from serving as sites of attachment for exogenous pathogens
  - No attachment = expulsion by the host
- produce antimicrobial factors that help to kill or limit the growth of pathogenic organisms (eg < salmonella)
- carry out a range of biochemical reactions that benefit the host eg. Intestinal microbes produce enzymes that break down food thereby aiding digestion
  - Breakdown bile acids to products imp. in emulsification of fats
  - Whole range of intestinal species produce vitamin K... needed for the Synthesis of prothrombin (enzyme in blood clotting)
  - Role in development of intestinal epithelium and GALT

Significance of normal microbiota emphasized by:

“Germ free” - gnotobiotic animals (GA)
Delivered by caesarian section and maintained in special isolators
Free from detectable viruses, bacteria & other organisms
Two observations:
GA lived 2x longer than conventionally bred animals
Major COD differed
- infection killed conventional animals
- intestinal atonia frequently killed GA
In GA:
- Alimentary lamina propria is underdeveloped
- Little to no Ig is present in saliva or secretions
- Intestinal motility is reduced
- Intestinal epithelial cell renewal rate is half that of conventional
  - may be vitamin deficient
  - digestive systems do not function properly

Administration of antibiotics suggest microbiota protects from pathogens

Streptomycin administered to reduce normal flora in mice
Challenged with Strep -resistant Salmonella typhi
(normally requires 10
6 organisms establish GI infection)
In Strep treated animals, <10 organisms induced disease
Why?
Acetic/butyric acids usually formed as fermentation products of normal microbiota inhibits growth of S. typhi

Patients on broad spectrum antibiotics
Enteroscosis due to overgrowth of Cl. difficile
Candidiasis due to overgrowth of Candida sp.
Environmental infection by Ps. aeruginosa

More or less....on the microbiota

• Not all microbiota have been identified
  - unknown how many sp. we harbour
• microbial communities so complex, difficult to cultivate
  - estimated that fewer than half of microbes present have been identified
• know little about the interactions between organisms & the cells and tissues to which they attach
• little known about how microbiota are maintained
• more attention placed on disease inducing rather than the harmless....so less explored

Normal microbiota

Types of bacteria found associated with an individual vary enormously from site to site within the individual
therefore necessary to discuss biota of a particular site
variations arise as a result of differing selective environments at a site
chemical
physical
biological
mechanical
produce unique environment that selects which bacteria survive & grow
different microbes predominate at different sites during growth & maturation
The skin

Features of the skin:
- Skin is a readily accessible organ for bacterial colonization.
- Constantly in contact with large variety of bacteria from the environment and from other anatomical sites like RT and GIT.
- Skin surface is not hospitable to microbes; consists of dead cells (dry) and is slightly acidic.
- Some microbes can colonize skin surfaces & tend to be neutral or benign; many of these are transients (not survive very long).
- CF residents are able to grow and establish themselves there.
- Body keeps the numbers on the skin limited; varies with location of the skin surface (armpit, perineum, forearm, back).

Principal source of nutrients for skin microbes are sweat and sebum.
- Distribution of hair and sebaceous glands varies across skin.
- Armpits (enclosed, hairy, moist) support a denser population ($10^6$/cm$^2$) than the back ($10^2$/cm$^2$).
- Dry surface of skin generally supports < moister sweat & hairy regions.

Successful skin colonizers:
- Able to adhere to keratinized epithelial cells.
- Able to utilize lipids as a carbon and energy source.
- Able to tolerate high salt concentrations.
- Staphylococcus, Micrococcus, Propionibacterium, Corynebacterium (Gram positives).

Skin microflora can induce disease:
- Staph. aureus: transient from the nose; boils, wound infections, food poisoning.
- Staph. epidermidis: infections of prosthesis devices & implants as biofilm; highly resist antibiotic-endocarditis.
- Propionibacterium acnes: causes acne in adolescence and young adults.

The oral cavity

The oral cavity contains varying habitats:
- > 500 sp. identified so far.
- Total number in oral cavity estimated at $10^{10}$.
- Teeth, buccal mucosa, tongue, gingival crevice differ in nutrients, oxygen content, redox potential, pH.
- Teeth unique as non-shedding surface; form biofilm = dental plaque.
- Biofilms typically contain $10^{11}$ bacteria/gm wet weight.
- Bacteria in mouth constantly subjected to mechanical forces: swallowing, tongue movements, chewing.
- So ability to adhere to oral surfaces or already adherent bacteria an essential requirement to colonize the oral cavity.

Development of teeth in a child:
- New emerging tooth surface: S. sanguis & S. mutans.
- Buccal epithelial surface & gingival crevice: S. salivarius.
- Mostly lactic acid aerotolerant anaerobes attach to thin layer of salivary glycoproteins on teeth.
- Mouth predominantly Strep. spp.
- Also colonize the tongue and inner cheek.
- Dental extraction results in transient bacteriemia (Strep. Spp.) which can develop into endocarditis.
- S. pneumoniae carried by 25% population in the mouth or throat.
- Not as successful as other Strep's in the mouth.
- May cause otitis media in children and in severe cases of influenza, is a 2' infection: pneumonia.

The gingival area

Normal colonization by a mixture of Gm+ and Gm- bacteria other aerotolerant or obligate anaerobes.
- Gingival bacteria form plaque on the root surfaces of the teeth if plaque growth continues, becomes more Gm-ve and spirochetes may appear.
- This new population produces proteases-destroy gum tissue;
- Bleeding gums- receding gums- tooth loss.
- Periodontal disease affects 80% population in the Western world induced by Gm-ve anaerobic rods and spirochete (T. denticola).
- Yeast: candida albicans minor in the mouth and usually benign;
- Causes oral thrush in antibiotic treated, immunocompromised, cancer, AIDS in children whose oral biota not yet fully developed.
Normal microbiota of respiratory tract

Respiratory Tract

Respiratory tract inhales >10,000 bacteria per day either freely or as particulate matter. Mechanisms to reduce pathogens gaining access include hairs in nostrils that trap and remove large particles. Mucociliary escalator trap particles that get through the hairs. Mucus itself traps particles and bacteria; in the larynx they can be swallowed or coughed.

Resident microbes need to overcome:
- resistant expulsion
- able to adhere to epithelium lining the RT

These mechanisms include:
- lysozyme
- lactoferrin
- secretory IgA
- complement

These are:
- Strep spp.
- Staph’s
- Corynebacterium spp.
- Gm-ve cocci

Nasopharynx

Haemophilus influenzae (capsule... meningitis, pneumonia, acute epiglottitis)
- Only present in 4% of the population
- Moraxella catarrhalis
- Neisseria spp. (10% population harbour N. meningitidis)

Oropharynx

Strep. spp (α-haemolytic) predominate
- Haemophilus sp.
- Neisseria sp.
- Mycoplasma
- 10% population harbour S. pyogenes (β-haemolytic)
- Causes pharyngitis which progresses to rheumatic fever or glomerulonephritis
- Also causes impetigo, cellulitis

Lower respiratory tract
- Usually sterile due to mucociliary escalator, alveolar macrophages

The nose

Predominantly Gram +ve organisms, some of which can cause disease:
- S. aureus
- S. epidermidis
- Strep. Pneumoniae
- Diptheroids (Corynebacterium spp.)
- Staph. aureus

Transferred from nose to the skin when handled food

1/3 S. aureus strains produce enterotoxin which if ingested causes vomiting and cramps; rarely fatal but unpleasant.

Now gloves must be worn by food handlers.

The Gastrointestinal Tract

Comprises most of the bacteria inhabiting humans ($10^{14}$) with a mass (1 kg) and colonizing GIT surface area of ~200 m$^2$.

Tract environment:
- Very little ingress of air: predomin. anaerobic; low redox potential
- Enormous range and availability of nutrients for bacteria to thrive
- Tract consists of number of fluid filled cavities so ability to adhere to mucosa is not essential
- Proteolytic enzymes, bile salts & mucosal surfaces are antibacterial mechanisms in the tract
- Stomach acidity and pepsin allow fewer organisms to enter intestines

Duodenum and jejunum
- Acidic at pH 4.5
- Sparse microbiota $10^5$/mL but more complex than the stomach

GIT - normal biota
The stomach

- Usually few (10^3/mL) due to acid contents of stomach and action of pepsin
- Helicobacter pylori may be present in up to 80% population by age 10
- causes gastric cancer and peptic ulcers in some who harbour it
- exceptions when movement through the stomach is rapid

Helicobacter pylori

- Mainly members of acideric genera (Strep and Lactobac.)
- May be present in up to 80% population by age 10
- Causes gastric cancer and peptic ulcers in some who harbour it
- Exceptions when movement through the stomach is rapid or microbes resistant to gastric acid
- Intestinal obstruction, gastrectomy may flush duodenal contents up
- Acid barrier is not intact in neonates
- Result in biota like oropharynx + Gm-ve of GIT

Ileum - next region of small intestine

- More 10^9/mL and complex organisms
- Lactobacillus, Bifidobacterium, Enterococcus, Bacteroides, Veillonella, Clostridium and E. coli

The Colon

- Large numbers (10^9-11/mL) attached to mucosal surface of the colon
- pH of this region is neutral and low in oxygen
- Nearly 500 species isolated from the colon; 40 sp. Common
- Bacteroides sp. reg. comprises 10% microbiota
- Obligate anaerobes comprise >90% (10^10 cells/g intestinal content)
- Five common genera:
  - Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, Fusobacterium
- Regularly isolated but less frequent:
  - Escherichia, Enterobacter, Proteus, Lactobacillus, Veillonella

- The Colon - large intestine

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- The Colon

- Holding tank for bacteria, similar to cattle rumen
- Neonates whose colons are free of bacteria at birth, first colonized by O2 utilizing E. coli; once established, render colon anoxic to permit anaerobes like Bacteroides to colonize

- Takes ~2 years for a child's colonic state to stabilize
- Infant's stomach is not as acidic as an adult's allowing more ingested bacteria into the intestine alive

- Period during microbiota development is window of opportunity to pathogens
- Clostridium botulinum spores (honey), pass harmlessly; adult colon as cannot compete with adult colon microbiota cf. infant; less competition
- Spores germinate --- produce toxin --- into colon = fatal paralytic botulism
- Good example of protective role of microbiota

- Common colon residents that cause disease

- Clostridium perfringens - gas gangrene
- Bacteroides spp - perforation, intra abdominal abscess
- Clostridium difficile - pseudomembranous colitis
- E.coli - diarrhoeal diseases, UTI, neonatal meningitis

- Example. Pseudomembranous colitis
- First observed with introduction of antibiotics
- Broad spectrum antibiotics can reduce anaerobes in colon
- Results in overgrowth of Clostridium difficile (5% harbour it; kept low by biota)
- And toxin production
- Toxic produces severe damage to colon lining ------- death in days

- Indigenous GIT microbiota can prevent infections

- Mechanisms:
  - Production of bacteriocins
  - Microbial competition for nutrients
  - Inhibitory effect of fatty acids produced by anaerobes on the growth of Salmonella typhimurium
  - Shigella sp
  - Pseudomonas aeruginosa
  - Klebsiella pneumoniae

- Mechanisms by which the normal flora compete with invading pathogens
The urogenital tract: urethra and bladder

- Regularly flushed by sterile urine—no microbiota
- Except for distal portion of urethra, sim. to skin (in males)

**Females**
- Distal urethra colonized by skin, anal and vaginal microbiota
- Pre-puberty & post-menopausal—alkaline vaginal secretions
  - Main microbes are Staph sp. and Strep sp.
- Between puberty & menopause—acidic (pH 4-7) vaginal secretions
  - Due to fermentation of glycogen which accumulates in epithelia due to oestrogens
  - Low pH encourages Lactobacilli sp., constant dominant microbiota -vagina

Opportunistic Infections

- Clinical conditions that may be caused by normal microbiota

**What changes cause a switch from mutualistic /commensal to disease associated parasite?**

1. Damage to epithelium: - burns, wounds, bites
2. Presence of a foreign body
3. Transfer of microbiota to unnatural sites
4. Suppression of the immune system by drugs or radiation
5. Impairment of host defences due to infection by exogenous pathogens
6. Disruption of normal microbiota by antibiotics

**2. Presence of a foreign body**

- Advances in surgery and science of biomaterials: artificial prostheses, heart valves, implants
- Catheters into body orifices and in skin remaining for periods of time
- Biomaterials unlike epithelium do not have a shedding surface allowing accumulation of bacteria in a biofilm
- Biofilm=adherent aggregate of microbes
  - less susceptible to phagocytosis
  - less accessible by antibiotics
  - less susceptible to serum products
- Medical devices also interfere with blood and lymphatic flow in neighbouring tissues rendering the host less able to cope with adherent microbes
- Also interfere with urine flushing and mucociliary escalator in URT
- Organisms involved varies with the site
  - Staph aureus, Staph epidermidis, Candida albicans, Ps. Aeruginosa
- Iatrogenic=diseases that result from a medical procedure

**3. Transfer of microbes to “unnatural” sites**

- Close proximity of colon to urethra in females facilitates colonization
- Of peri-urethral area by colonic microbes
  - E.coli, Proteus spp. Klebsiella spp.
- Ascend urethra—bladder=UTI
  - E. coli most common in women between 20-40 years of age
- Lower respiratory tract—usually sterile
  - Oral microflora gain access
  - An individual loses consciousness
  - Tubes are inserted
  - Food/gastric fluid is inhaled
- Presence of anaerobic members of oral microbes in LRT
  - aspiration pneumonia (most common COD in elderly)
- Disease is polymicrobial—anaerobes, Gm-ve bacilli, Gm+ve cocci

**4. Suppression of the immune system by drugs or radiation**

- Cancer therapy involves use of cytotoxic drugs and radiation
- Effect is to kill rapidly dividing cells
- Side effect:
  - kills neutrophils, constitutive defence against bacteria
  - Depressed antibody production
  - Impaired complement function
  - weakened ability to deal with infections
- Transplant patients=immune system depressed
- Prone to infection by a wide variety of microbes:
  - Candida sp. E.coli, Staph. Aureus, Ps. aeruginosa
- These infections often acquired whilst in hospital from medical staff or personnel or equipment=nosocomial infection
- Most hospitals have nosocomial rates of 5-10% of inpatients
5. Impairment of host defences due to infection by exogenous pathogens

Common example is influenza infection

- Destroys cells lining the URT and LRT leading to an impairment to exclude bacteria by epithelium
- Inhibits phagocytosis by alveolar macrophages
- Enables survival of S. aureus, Strep. Pneumoniae, H. influenzae
- Which can result in fatal pneumonia

HIV infection: Causative agent of AIDS

- Destroys key component of immune system - CD4 T lymphocytes
- Vulnerable to all sorts of opportunistic infections esp. by normal microbiota
  - Candida sp.
  - Strep. Pneumoniae
  - Corynebacterium sp.
  - Herpes infections plus many more

6. Disruption of normal microbiota by antibiotics

- Microbes usually inhabiting a particular anatomical site consist of complex community controlled by interactions amongst microbes present
- Competition for adhesion sites & nutrients
- Interdependence - food webs
- Production of bacteriocins etc...

- Treatment with antibiotics - dramatic effect
  - Encourage overgrowth of the subdominant resistant species

- Result: Organism present in low numbers may become dominant and be able to initiate infection
  - Tetracycline
  - Ampicillin, clindamycin, cephalosporins treat Gm negative
  - Permit overgrowth of Gm +ve Cl. difficile produces toxin - diarrhoea dis. = pseudomembranous colitis

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**Immunology - Levels of Defense**

**First line**
- Cellular factors - Phagocytosis (chemotaxis, adhesion, ingestion)
- Opsonins ie. C3b, CRP, antibodies - Lead to phagocytosis and phagosome-lysosome formation
- Natural killer cells

**Second line**
- Humoral factors - complement, acute phase proteins, lysozyme, coagulation, fibrinolysis, kallikrein systems
- Serologic - B cells and antibodies

**Fourth line**
- Cell-mediated immunity - T cells (helper and cytotoxic) and cytokines

**Protection from Infectious Agents**

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Non-specific Host Defences

• Inflammation
• Sneezing
• Filtration of air including turbulence
• Cough
• Vomiting
• Diarrhoea
• Itching
• Fever

Innate Immunity

Mediated by cellular & chemical mechanisms
Non specific & always present
Has to be activated
Result is inflammatory

Inflammation is a process which always produces A measure of damage to the host—scarring

Define: Inflammation

Origin: L. Inflammatio, inflammare = to set on fire

1. A localised protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue.

Cardinal Signs
1. Pain (dolor)
2. Heat (calor)
3. Redness (rubor)
4. Swelling (tumour)
5. Loss of function (functio laesa)

Histologically
1. Dilatation of arterioles, capillaries and venules
2. Increased permeability and blood flow
3. Exudation of fluids, including plasma proteins
4. Leucocytic migration into the inflammatory focus

The Inflammatory Response

1. Antibody Independent
   (a) Tissue Injury
   (b) Alternate complement pathway
2. Antibody Dependent
   (a) Classical complement pathway
   (b) Mast cell degranulation

Chemotaxis

1. The process of directed cell migration, which is a dynamic and an energy-dependent activity.
   - Initial recruitment of macrophages depends largely on C5a and arachidonic acid metabolites, whereas following injury, the prolonged recruitment from 6 to 48 hours is mediated by the production of chemotactic cytokines

Activation of mediators of inflammation

Earliest event is activation of complement

Complement system is comprised of 30 proteins in serum and tissues

Complement cascade is ordered sequence
- Induced by whole microbe (alternate pathway)
- Induced by antigen-antibody complexes (classical pathway)
Both result in MAC......lys...=death

Complement
Also responsible for leukocyte migration to site of microbial invasion
By chemotactic factors
- Most important is C5a

C3b is most important opsonin
Can also bind platelets & release other mediators of inflammation

Define: Chemotaxis

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Chemotaxis

- Exogenous mediators
  - N-formylmethionine terminal amino acids from bacteria
  - Lipids from destroyed or damaged membranes (including LPS)
- Endogenous mediators
  - Complement proteins (C5a)
  - Chemokines, particularly IL-8
  - Arachidonic acid products (LTB4)

Polymorphonuclear neutrophil-PMN

- Phagocytic cell-main line of constitutive defence
- Microbe gains entry beyond epithelial surface
- Next in line are phagocytic PMN
- Specialize in killing extracellular microbes
- PMN - nucleus is multi lobed
- Circulate in blood
- Short lived but numerous
- Produced in bone marrow
- Generally first to arrive at site of microbe invasion
- Attracted by chemotaxis (C5a)...
- Then phagocytose...& kill microbe
- Die in battle & form pus

Cells of the Immune System

- Polymorphs and macrophages are relatively primitive phagocytic cells, and are part of the non-specific response to pathogens (innate immunity, natural immunity).
- Macrophages also have specialized antigen-presenting functions in the specific response to pathogens and antigens (acquired immunity).

Activities of Inflammatory Mediators

- Vasodilatation - histamine, C5a, kinins
- Permeability - histamine, C5a, kinins, leukotrienes
- Neutrophil chemotaxis - C5a, leukotrienes, chemokines, PAF
- Neutrophil activation - PAF, TNF, IL-1
- Endothelial activation - IL-1, TNF
- Opsonisation of bacteria - C3b, antibodies
- Coagulation - PAF, IL-1, TNFα
- Entrapment of bacteria - fibrin

Bactericidal Activity

- Activated oxygen species
  - Superoxide (\(\text{O}_2^\cdot\)) - formed via NADPH oxidase
  - Hydrogen peroxide (\(\text{H}_2\text{O}_2\)) - formed via spontaneous dismutation of superoxide
  - Hypochlorous acid (HOCl) (Myeloperoxidase) - probably the primary bactericidal agent in neutrophils; myeloperoxidase converts \(\text{H}_2\text{O}_2\) into HOCl
  - Hydroxyl radical (\(\text{OH}\))

Leukocyte Extravasation and Phagocytosis

- Margination, rolling, and adhesion
- Transmigration (diapedesis)
- Migration toward the site of injury along a chemokine gradient
PMN Chemotaxis

Role of C5a is to attract PMN to site by diffusing away from it PMN’s respond by stopping their rolling motion and sticking to A blood vessel wall where C5a concentration is highest Then proceed to push endothelial cells apart and enter by Transmigration to C5a conc. Gradient C5a & cytokines stimulate PMN's to become activated & better able to phagocytose bacteria

C3b as opsonin
Is a sticky molecule Binds to PMN surface & bacterial surface Opsonin helps PMN to ingest bacteria Cannot bind to human tissue (sialic acid)

Complement Cascade

- 11 proteins - C1-9; C1 = 3 subunits (q,r,s)
- Classic and Alternative/Properdin pathways
- Classic = C1 binds Ab + Ag complex
- Alternate = recognises poly-fructose/glucose
- C3 is the critical control point, and interacts with both pathways
- C3b leads to bacterial opsonisation
- C3a and C5a are known as anaphylotoxins, and are capable of releasing histamine from mast cells, along with potent chemotactic abilities (C5a)
- Membrane attack complex (MAC) is the active agent of complement lysis and consists of C5-9
**Biological Functions of Complement**

**Cytokines**

**General Properties of Cytokines**
- May be produced by several cell types
- Induce effects via autocrine, paracrine, or endocrine mechanisms
- Bind to specific high-affinity receptors and affect cells via transduction mechanisms