Normal Microbiota & Innate Immunity

Infectious Diseases Modules

1. Overview
2. Normal microbiota & innate immunity
3. Host defences in infection
4. Examples of infectious diseases
5. Bacterial pathogenesis- virulence
6. Bacterial pathogenesis- genetics
7. Bacterial pathogenesis- methods
8. Paradigms of microbe-host relationships
9. Viruses
10. Mycoses and animal parasites
11. Medicine and Infection
12. Future challenges in infectious diseases

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

Barriers to Infection

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

Barriers to Infection

First barriers to cross for any infectious agent to the normally sterile areas of the body are:

The skin

Conjuctivae 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

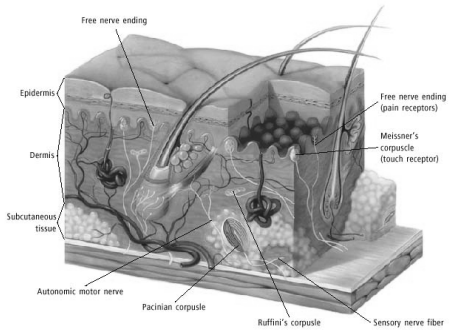
Generally surface is dry & acidic; not microbe friendly

Viruses cannot replicate in dead cells

Dead cells of skin, constantly sloughing off plus anything attached to it,eg. Microbes

see fig 12.2 from recommended textbook

Epithelium - Skin



Skin barriers to infection

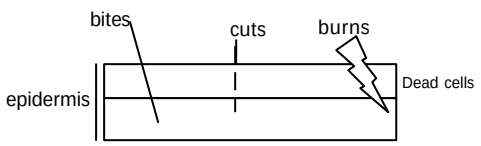
As the cells of the dermis grow out - into epidermis produce high levels of keratin - not utilized readily by microbes

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



Breaches in skin show significance of the barrier
Cuts, burns, bites, trauma allow surface or environmental bacteria into the tissue.....cause infection

Burns destroy specific and non specific defences by destroying the tissue.....
1º may survive burn trauma.... ? Survive 2º 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 protien that form holes in microbial membranes

Mucin is constantly being shed and replaced so trapped microbes Constantly expelled from the body

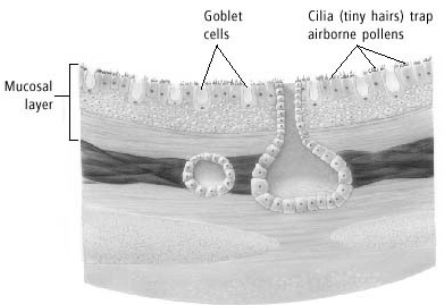
Epithelial cells also replaced frequently,
so any attached microbes that get through mucin will be shed.

Phagocytes in MALT and GALT will engulf and destroy invaders

Sloughing of mucin layer

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

Mucosal Epithelium



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 the mucociliary escalator directs the flow of secretions up to oropharynx...swallowed

Smallest particles <5uM are ingested by alveolar macrophages

Normal flora also protects against colonization

Respiratory Tract

- Constant exposure to thousands of potential pathogens

Unique defence structure:

- **Mucociliary escalator**
  - Particles >5micron : 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

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

Alimentary Tract –barrier to infection

Constant swallowing acts to flush microbes into stomach

Normally acidic stomach eliminates majority of ingested microbes

In 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

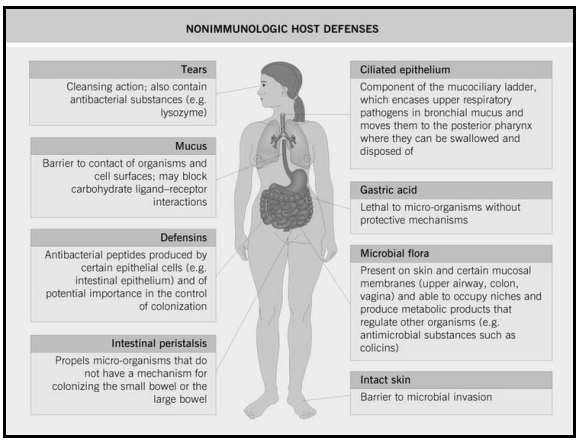
The Eyes

Protective barrier is flushing by tears

Tears have lysozyme- lyses cell walls of microbes

Chemical Epithelial Barriers

•Enzymes	Lysozyme (tears, saliva, sweat) Pepsin (stomach)
•Acid/Base	Fatty Acids/amino acid (skin) Gastric acids (stomach)
•Antimicrobial	Transferrin (mucus), Defensins



Normal microbiota of the human body

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-lower pH & blocking colonization by more dangerous pathogens

E. coli synthesizes vitamin K and some B vitamins that are absorbed int o the blood stream 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
>1000's species bacteria, funghi, live symbiotically on the human body

External surfaces: skin and conjuctiva 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

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 conventionals
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
Enterocolitis due to overgrowth of Cl. Difficile
candidiosis 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

therfore 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 & from other anatomical sites eg 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 which 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)

The skin

Principal source of nutrients for skin microbes are sweat and sebum

distribution of hair and sebaceous glands vary 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:
be aerobic, fac. Anaerobe, anaerobe
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
infective 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
differing 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
- constant flow of saliva
- swallowing
- tongue movements
- chewing

so ability to adhere to oral surfaces or already adherent bacteria an essential requirement to colonize the oral cavity

The oral cavity

Development of teeth in a child:
new emerging tooth surface- S. sanguis & S. mutans
buccal epithelial surface & gingival crevice -S. salivarus
mostly lactic acid areotolerant 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 bactereamia (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 2^0 infection....pneumonia

The gingival area

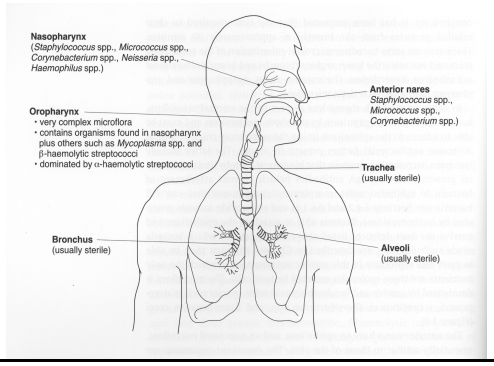
Normally colonized by a mixture of Gm+ and Gm- bacteria
either aerotolerant or obligate anaerobes

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 Western world
induced by Gm-ve anaerobic rods and sirochaete (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 >10000 bacteria per day either freely or as particulate matter
mechanisms to reduce pathogens gaining access
hairs in nostrils-trap & remove large particles
mucociliary escalator trap particles that get through the hairs
mucous itself traps particles and bacteria.....larynx (swallowed, coughed)

Resident microbes need to overcome:
resist expulsion
able to adhere to epithelium lining the RT
overcome lysozyme, lactoferrin, secretory IgA and complement

these are Strep spp. Staph's , Corynebacterium spp, Gm-ve cocci

Nasopharynx

Haemophilus influenzae (capsule...meningitis, pneumonia, acute epiglotitis) only present in 4% 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....progresses to rheumatic fever or glomerulonephritis
also causes impetigo, cellulitis
up to 70% harbour S. pneumoniae (meningitis, pneumonia, earache)

Lower respiratory tract

Is usually sterile due to mucociliary escalator, alveolar macrophages

The nose

Predominantly Gram +ve
some of same organisms as the skin
S. aureus
S. epidermidis
Strep. Pneumoniae
diptheroids (Corynebcaterium spp.)

Staph aureus
transferred form nose to the skin
transferred from nose to food handler to 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

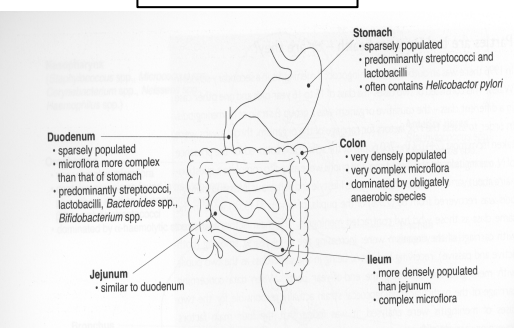
Comprises most of the bacteria inhabiting humans (10^{14}) with a mass (1kg) and colonizing GIT surface area of ~$200m^2$

Tract environment:
-very little ingress of air: predom. 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 not essential
-proteolytic enzymes, bile salts & mucosal surfaces are antibacterial mechanisms in the tract
-stomach acidity and pepsin allow few 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
Mainly members of acideric genera (Strep and Lactobac.)

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
or microbes resistant to gastric acid....mycobacteria

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 sm intestine

More 10^9/mL and complex organisms

Lactobacillus, Bifidobacterium, Enterococcus, Bacteroides, Veillonella, Clostridium and E. coli

The Colon- large intestine

Large numbers (10^{9-11}/gM) attached to mucosal surface of the colon
And are present in the lumen
-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/gM intestinal content)
Five common genera:
Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, Fusobacterium
Regularly isolated but less frequent:
Escherichia, Enterobacter, Proteus, Lactobacillus, Veillonella

The Colon

Holding tank for bacteria, similar to cattle rumen
Neonates whose colons are free of bacteria at birth, first colonized by O_2 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
Clost. 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

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

Example. Pseudomembranous colitis
First observed with introduction of antibiotics

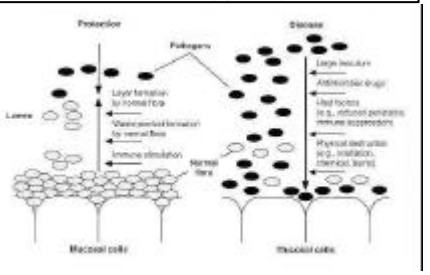
Broad spectrum antibiotics can reduce anaerobes in colon
Results in overgrowth of Cl.difficile (5% harbour it; kept low by biota) and toxin production
Toxin produces severe damage to colon lining-------death in days

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 fermentaion 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
1. Disruption of normal microbiota by antibiotics

2. Presence of a foreign body

Advances in surgery and science of biomaterials:
---artificial prostheses.....heart valves, joints, 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
(1) An individual loses consciousness
(2) Tubes are inserted
(3) 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. aures, 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 includes:
Competition for adhesion sites & nutrients
Interdependence –food webs
Production of bacteriocins etc...

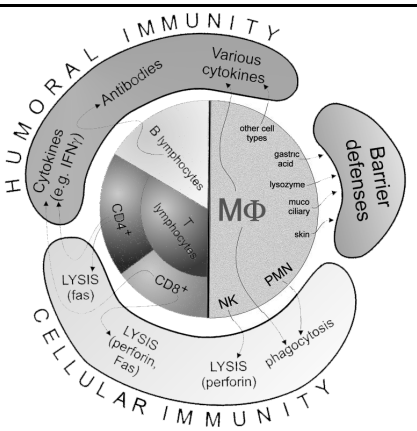
Treatment with antibiotics –dramatic effect
Encourage overgrowth of the subdominant resistant species

Result=organism present in low numbers may become dominant and be able to initiate infection

Tetracycline
Permits overgrowth of resistant *candida* in mouth===thrush

Ampicillin, clindamycin, cephalosporins treat Gm negative
Permit overgrowth of Gm +ve *Cl. difficile*---produces toxin---diarrhoea dis. =pseudomembranous colitis

Innate Immunity



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, fibrinolysin, kallikrein systems

Third line
- Serologic - B cells and antibodies

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

Protection from Infectious Agents

Innate Immunity
- Fever
- Interferon
- Neutrophils
- Macrophages
- NK cells

Adaptive Immunity
- B cells
- T cells

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

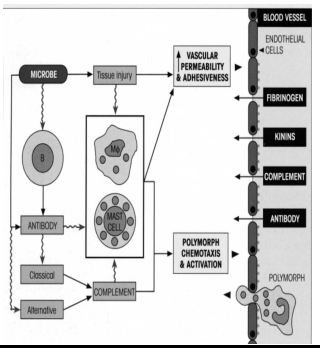
Origin: L. Inflammatio, inflammare = to set on fire

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

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.....lysis.....=death

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

Other complement components act as opsonins
-bind to microbe
-facilitate uptake by phagocytosis & removal by macrophage
C3b is most important opsonin
Can also bind platelets & release other mediators of inflammation

Define: 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 the production of chemotactic cytokines

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 (LTB_4)

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 ($^{\bullet}O_2$) - formed via NADPH oxidase
- Hydrogen peroxide (H_2O_2) - formed via spontaneous dismutation of superoxide
- Hypochlorous acid (HOCl) (Myeloperoxidase) - probably the primary bactericidal agent in neutrophils; myeloperoxidase converts H_2O_2 into HOCl
- Hydroxl radical ($^{\bullet}OH$)

Leukocyte Extravasation and Phagocytosis

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

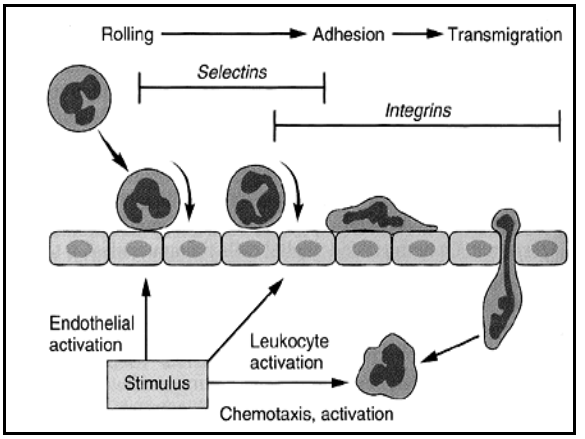
PMN Chemotaxis

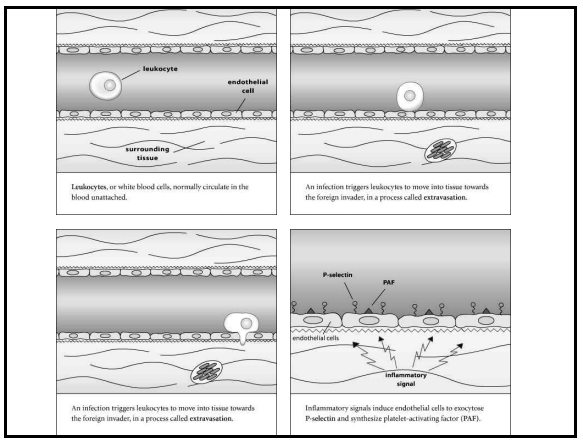
Role of C5a is to attract PMN to site by diffusing away from it
PMN's respond by stopping their rolling motion and sticking to
A blood vessel wall where C5a concentration is highest

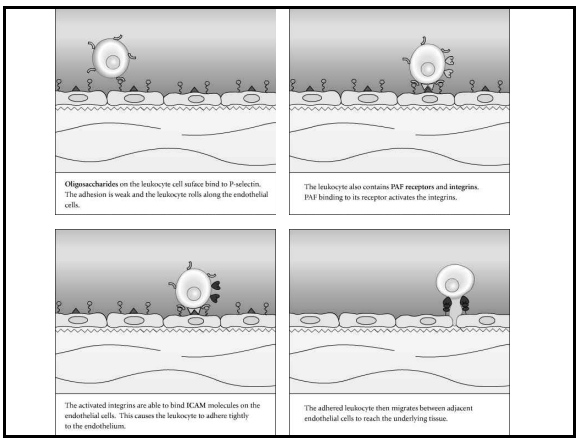
Then proceed to push endotheleial cells apart and enter by
Transmigration to C5a conc. Gradient

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)

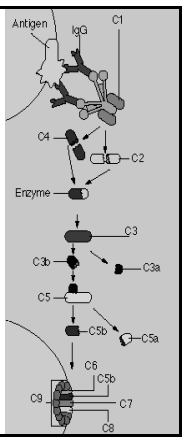




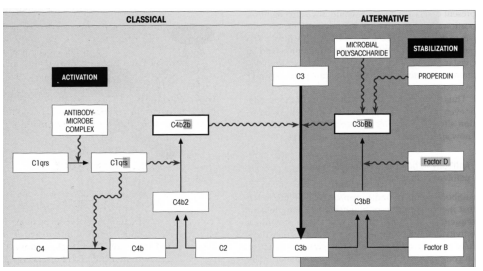


Complement Cascade

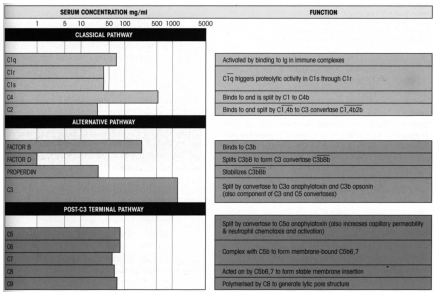
- 11 proteins - C1-9; C1 = 3 subunits (q,r,s)
- Classic and Alternative/Properdin pathways
- Classic = C1 binds Ab +Ag complex
- Alternate = recognises poly-fructose/-glucose
- C3 is the critical control point, and interacts with both pathways
- C3b leads to bacterial opsonisation
- C3a and C5a are known 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



The Complement Pathway



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