8. Control of Microorganisms –sterilization and disinfection.

It is useful to first learn the definitions of some basic terms when we discuss control of microorganisms;

Sterilization is an all or nothing event, something is either sterile or it is not, there is no such thing as *almost* sterile or *partly* sterile (as with the incorrect use of expressions such as "quite unique", something is either unique or not – there are no degrees of uniqueness). Sterility refers to the complete absence of life, it is a process that kills or physically removes all living things from an object or material.

Disinfection, sanitization, antiseptic are not terms that refer to sterility! These are all terms that refer to various *degrees* of absence or killing of microorganisms in particular situations. Disinfection is the use of a chemical agent (a disinfectant) to kill microbes on or in inanimate objects in order to reduce the numbers of pathogenic organisms so that there is no or a much reduced threat of disease, most disinfectants are not safe for use on human skin. An antiseptic is a chemical agent that can be used safely on tissue surfaces (skin usually) to kill microbes or inhibit their growth, but cannot be safely taken internally. A sanitizer is a chemical agent usually used on food handling equipment to reduce bacterial numbers, and the term is also used for the process of washing the hands with soap and water.

I will consider how organisms are killed or reduced in number on objects such as walls, medical equipment, in solutions and so on, by use of chemicals or heat.

Either **physical** means or **chemical** means are used to control microbial growth on or in objects. Most often the physical agent used to kill microorganisms is heat, but radiation is also used, and we can also include freezing and drying as physical methods. Chemical control of microbial growth on or in objects is generally through highly toxic agents that therefore can be used in low concentration, but these are generally not suitable for medical use.

Chemical agents.

I am not referring here to antimicrobial agents used to treat infections, this is dealt with later. I am dealing here with chemicals used on or in *inanimate* objects.
There are a number of tests that can be used to test the efficacy of chemical antimicrobial agents such as the phenol coefficient, the filter paper method, and use dilution tests.

The **phenol coefficient** sets the chemical **phenol** (very nasty stuff in its pure form, it is poisonous and also causes severe burns) as equal to 1.0, as the standard, when used as a microbial control agent, and other chemicals are prepared at dilutions in test tubes which approximate their usage concentrations and are compared against dilutions of phenol for their ability to kill microorganisms or prevent their growth. Calculations can be done to compare the effectiveness of the tested agent against phenol. A phenol coefficient of less than 1.0 means that the tested agent is less effective than phenol, a coefficient of 1.0

means it is as effective as phenol, greater than 1.0 means it is more effective than phenol. Thus an agent with a phenol coefficient of 3.0 is three times as efficient as phenol as a control agent, and if it had a coefficient of 0.3 it would be judged only a third as effective as phenol at safe use levels. This phenol coefficient test is useful and has been performed for many years, but it is somewhat arbitrary in that the comparison of chemical agents that differ in structure and basis of activity from phenol is poorly understood.

Chemical agents can be dropped in solution on to **filter paper discs** and placed on agar plates which have a lawn of test bacteria and inhibition of growth can be assessed, this can be done quantitatively, I will describe this in class. This is called the **Kirby Bauer** method when it is used to assess the suitability or sensitivity to antimicrobial agents used to treat human infections

Selection of a disinfectant. The criteria for choosing the best disinfectant are – they should be fast acting, have a wide range of effectiveness, be penetrating, be easy to use and stable, are inexpensive and easily available, and are pleasant to work with.

How chemical agents act:

They damage protein structure - acids, alkalis, oxidizing agents, some heavy metals.

Acids and alkalis can be used to control microbes, sometimes they work by destruction of microbial cells, or they work because they create extremes of pH in which microbes cannot grow - as with the use of vinegar in canning of vegetables to prevent bacterial growth, acidic conditions are commonly used to deter microbial growth but, as far as I know, alkaline conditions have not.

Metals
Silver metal damages proteins and is used in ceramic water filters to keep bacterial growth from occurring that infiltrates the filtering medium, silver nitrate drops are (or at least were) used in the eyes of new born babies to prevent infections, mercury salts are powerful antimicrobial agents used in products such as vaccines. This ability of metals like copper and silver to kill microbes is called the **oligodynamic** effect, generally what happens is that the metal ions combine with the sulfhydryl groups of the microbial proteins and disrupt their structure – denaturation occurs.

They damage cell membranes - (surfactants - wetting agents) - soaps and detergents, also other chemicals such as phenol. Soaps and detergents affect cell membranes and also act physically to loosen deposits and wet affected areas so that agents reach contaminated areas more easily.

Positively charged (cationic) detergents are used to sanitize food utensils and surfaces, quaternary ammonium compounds (“quat’s”) are an important example and are also extensively used in industrial systems. However, some *Pseudomonas* pathogens can actively grow in quat’s, and quat’s can be inactivated by soaps and anionic detergents, and can also be inactivated by the fibres of gauzes and bandages. Quat’s are good killers

of gram positives, but not as effective against gram negatives, they are also fungicidal, amoebicidal, and virucidal if the virus is enveloped.

Negatively charged (anionic) detergents are used in laundry cleaning and household products, these include sodium lauryl sulphate, which is also found in toothpaste.

Alkylating agents and dyes affect other microbial cell components
These disrupt protein and RNA and DNA structure and function, they include formaldehyde, glutaraldehyde (used in industry to disinfect feedwater), propiolactone, and gaseous ethylene oxide (a very toxic explosive compound).

Dyes are often powerful antimicrobial agents and were amongst the first agents used to kill bacteria. Crystal violet is still used against some gram positive bacterial skin and mouth infections.

Halogens are powerful antimicrobial agents;

> **Chlorine** is the active ingredient in common bleach and is also injected in gas form into municipal water supplies. it is a powerful antimicrobial agent and is relatively cheap. Organic matter in water (such as humic substances from plant leaf matter) will bind chlorine and inactivate it, so more has to be used to offset this, and this results in the presence of organochlorine compounds in water which are a contentious environmental and health issue.
> **Iodine** is often used in areas where *Giardia* is a problem, since iodine can kill the parasite and its cysts, which chlorine cannot do efficiently when used at acceptable levels. It can be found in topical formulations – used on the skin, but caution has to be exercised when it is used to disinfect water, since iodine accumulates in the thyroid gland.
> **Bromine** is sometimes used in place of chlorine and is the active component of many of the water "purifying" tablets that are carried by campers. It was once used extensively as gaseous methyl bromide to fumigate soils - but this is now generally banned. Bromine is often used in swimming pools. Bromine is not used to disinfect municipal water supplies.

Phenols are commonly used as disinfection agents for surfaces, phenol was also the very first compound used to disinfect during surgery, related compounds are cresols, hexachlorophene, trichlosan.

Alcohols (methyl-, ethyl-, propyl-) are good surface disinfection agents and act also to wet surfaces so that agents dissolved in them penetrate microbial structures and slimes better, they are generally mixed with water which causes them to penetrate cells better and to wet surfaces more efficiently.

Physical destruction of microbes;

Heat.

DRY heat is used to sterilize surfaces, and materials which are not likely to break down in high heat and which do not contain any liquids. Glass Petri dishes and culture vessels, and metal surgical instruments are examples. Dry heat penetrates more slowly than steam heat, so higher temperatures and longer times are required, typically 160-170 C or more for several hours, depending on the distribution and the load of materials in the oven.

MOIST heat (steam). The classic **autoclave** is the prime example here, which is a sophisticated version of the pressure cooker used in home canning and bottling of foods. Moist heat penetrates more quickly than dry heat, and is used to sterilize culture solutions and agar preparations, and to sterilize surgical instruments etc. A prime reason for pressurized steam heat is that it is needed to kill bacterial endospores, which can withstand boiling. Typically a **pressure** of 15 psi (pounds per square inch) is needed to create steam at a high enough temperature (121 C) to kill endospores.

There are well developed procedures for loading autoclaves appropriately and cycling them for the correct period of time to perform correctly. One has to load and operate autoclaves properly, or it is possible for microbes to survive because they are inside a large volume of material and heat does not have time to penetrate all the way into the center. Special **indicator bacterial** endospores may be included in an autoclave to test that it reached proper sterilizing conditions at all points in the chamber. These bacterial endospores are inoculated into growth medium after being placed in an autoclave while it operates, if bacteria grow it means the autoclave did not attain the correct temperature needed to inactivate the endospores, so that they germinated and grew and reproduced to produce a visible hazy bacterial suspension in the broth, indicating an improper autoclave cycle. We will show you an example of this device during one of the tutorial sessions. A special indicator tape can be placed on items that enter an autoclave, they produce black stripes by a chemical reaction when 121 C is reached.

Do not ever be a “black box” personality when you are dealing with the killing of microbes, by this I mean that you should not think of any device used for heat killing of microbes as a “black box’, a device for which you need no intelligent consideration, something you just shove contaminated materials into and pay no heed to how much you put in, how much microbial load the material has, how hot the device actually has to get, how it has been loaded with materials, if you just allow yourself to lapse into regarding technology in this way, you could be courting disaster. Last year (2007) a number of hospitals in Western Canada had to stop doing surgeries, and patients who had surgery had to be tested for hepatitis and HIV because of improper sterilization of surgical instruments in an autoclave. Always make sure that the device is being used correctly.

There are, for instance, established measures that assist in planning the best procedures for heat killing given microorganisms in particular numbers and circumstances;

The **decimal reduction time** is the time in minutes that it takes for 90% of a given population of microorganisms to be killed at a given temperature. The **thermal death time** is the minimum length of time needed for given microbes in a given liquid to be killed at a given temperature. The **thermal death point** is the lowest temperature at which all microorganisms in a particular liquid will be killed in ten minutes. All of these values can be determined experimentally and used to provide information for the conditions needed when using heat to kill microbes whether in an autoclave in a hospital, or in industrial food processing operations.

Pasteurization is NOT sterilization. Solutions (milk is the best example) are heated at much gentler temperatures than in an autoclave for shorter periods of time. This reduces the microbial load and kills many pathogens but does not kill **all** bacterial pathogens and does not kill endospores. Pasteurization was originally developed by Pasteur to improve the shelf life of wine (he was under contract to a large group of French winemakers), but it does also make milk products safer to drink.

COLD retards the growth of microorganisms by slowing their metabolism, but it does not always kill them and some bacteria (like *Listeria*) and fungi do grow at near freezing temperatures. Refrigeration at 5 C retards the growth of many bacteria and fungi, freezing at – 10 to - 20 C (typical home freezer) is also an effective but not perfect means to retard microbial growth.

Drying preserves foods because microbes need water to grow and cannot get it on dried foods. Drying often just prevents growth of microorganisms, and does **not** always kill them. Freeze drying (**lyophilization**) is very effective at stopping growth of microbes.

Hyperosmotic conditions can preserve foods, because they cause water to be drawn out of bacteria and fungi so that they cannot thrive. Jam and pickles are classic examples because of their high solute loading – this makes jams and pickles highly hyperosmotic to the cytoplasm of bacteria and fungi which forces water to leave the cells by osmosis, but some microbes do grow in hyperosmotic conditions (some yeasts in brine pickles, or surface molds in jam for instance).

Hyper filtration can exclude microbes such as bacteria and fungi from solutions (but not viruses) and finds wide use for this purpose in the sterilization of delicate fluids which would be destroyed by other methods such as heat or irradiation, this is used in hospitals and research laboratories, but hyper filtration is expensive and not used much for bulk food preservation.

Radiation control of microbes:

Ultraviolet light has limited use in food preservation, it has poor penetration so it is used for surface sterilization, but it is becoming used in restricted cases to kill microbes in municipal water and to reduce the viability of microbes in sewage instead of using chlorine.

Ionizing radiation using X rays or gamma rays is an effective means for killing microbes. X-rays and gamma rays in particular are used to sterilize foods such as those used by astronauts or in packaged foods for the armed forces. There is a lot of contention about irradiation of food, it has become a public and political issue. Microwaves can kill microbes but it is not a good idea to rely on home microwave ovens to do this. There are effective microwave food irradiation devices that are used to prepare military "meals ready to eat" (MRE's) that are used by the US military and have long shelf lives. Microwaves often do not kill endospores, since they contain virtually no free water and free water is what is heated by microwaves to kill cells.

9. Control of microorganisms: Antimicrobial Therapy

Antimicrobial therapy is the use of chemical substances to control pathogenic organisms without causing harm to their hosts (us!).

Drugs, specifically **chemotherapeutic** agents; You will recognize the word **antibiotic** which literally means "anti-life" as being most commonly applied to the chemical agents taken by humans against microbial infections. Antibiotic is a term originally applied to agents that are synthesized by microorganisms, but is often used now to refer also to synthetic compounds generated or modified in the laboratory which are used to treat infections.

Of course, there are agents that can be safely used on our skin that could not be used internally, they are known as **antiseptics** and include many creams and lotions, some of them with a phenol compound base that is safe on the skin but toxic in the body.

I will use the term **antimicrobial agent** to avoid all of this confusion, I will apply this word to any chemical agent of any origin that is used to treat infections.

The first successful antimicrobial agents that were widely used were the **sulfa drugs** synthesized by Gerhard Domagk in 1932 from compounds related to the red dye prontosil, Domagk was initially not satisfied that prontosil, which he had found to be effective in mice experiments, would be as effective in humans, but his daughter contracted a deadly streptococcal infection, and Domagk, in desperation, gave her a dose of prontosil. She made a complete recovery (sadly, Domagk was later killed by an infection). Previously to the appearance of the first sulfa drugs, some arsenic based agents developed by Ehrlich (notably salvarsan) found limited use in treatment of syphilis. This disease (along with tuberculosis) decimated the population of Europe in the 1800's especially, and it was a disease that was rife in the upper classes and the intelligentsia. Many famous people had syphilis and died of it, for this reason desperate attempts were made to cure it, because many of the people who got syphilis could afford to pay for these measures. In some cases those who had syphilis were deliberately given malaria, because the very high fevers generated during the disease *sometimes* killed the syphilis bacterium, and one could live with malaria (since quinine could often cure or control it), whereas late stage syphilis was fatal and involved appalling symptoms, including insanity.

Next came **penicillin**, found by Alexander Fleming in the common fungus *Penicillium*, but it only became a useful practical affordable agent after the work of Florey and Chain, and some previous researchers had actually reported on the finding of this antibiotic property. Selman Waksman next isolated **streptomycin** form soil bacteria and **tetracycline** soon followed from the same source.

It is easy to kill microbes, there are many agents that can be used to do this, but killing microbes and NOT killing YOU, that is the hard thing to do. So - we have the notion of **specific toxicity**, we want an antimicrobial agent that attacks and kills microbes but does NOT harm or kill us.

The ideal situation is to have an antimicrobial agent that attacks cellular components that are found in microbes but not in us. In the case of bacteria this means attacking prokaryotic structures not found in eukaryotic cells. Numerous antibacterial agents do this. More of a problem exists when treating parasitic and fungal infections, since their cells and ours are eukaryotic.

Some antimicrobial agents have a **wide spectrum of activity** - they kill a wide range of types of microbes, some are of **narrow spectrum of activity**, they attack a smaller number of types of microbes.

In some cases an antimicrobial agent kills a bacterium (it is **bactericidal**), in some cases it does not kill it but prevents its growth (it is **bacteriostatic**).

Desired properties of antimicrobial agents;
They should be soluble in delivery fluids and in tissue fluids, they should have selective and stable toxicity, they should be non allergenic, and resistance should not be easily established. They should have a long shelf life, and a reasonable cost.

How do these antimicrobial agents kill bacteria or inhibit their growth?

We looked more fully at the graph of bacterial population life cycles earlier in the course. At this point you should understand that microbes are most vulnerable to attack by antimicrobial agents when they are actively growing and reproducing, and this is when they are growing **exponentially** in numbers, this corresponds to what is called **logarithmic** growth phase in the life cycle of a microbial population.

Antimicrobial agents that attack bacterial cell walls.
Bacteria have cell walls, our cells do not. (Eu)bacterial cell walls contain a unique polysaccharide polymer - **peptidoglycan**- which contributes to the strength and rigidity the wall needs to resist the high internal osmotic pressure of the cell. Penicillin and related agents interfere with the formation of peptidoglycan when the bacterial cell reproduces, this weakens the cell wall and the cell bursts (**lyses**). A component of tears and other body fluids (also found in egg white) an enzyme called **lysozyme**, also interferes with peptidoglycan synthesis and can cause bacteria to burst. Penicillin and other agents which have the same action are usually most effective against gram positive

bacteria, but often less so against gram negative bacteria because their peptidoglycan is protected by the outer membrane.

Peptidoglycan is emphasized here as a target of attack because it is such a crucial polymer for general medical bacteriology, peptidoglycan is a structural cell wall polysaccharide polymer unique to bacteria and provides rigidity and support against the high internal pressures of bacterial cells, without peptidoglycan bacterial cells would explode. This is why peptidoglycan presents such an attractive target against bacteria for those seeking to find ways to kill bacteria causing human infections. Human cells do not possess peptidoglycan, so if we can find drugs that can be used to inhibit peptidoglycan synthesis there is much less likelihood that the drugs will harm humans. This is what penicillin and related antibiotics do, they inhibit peptidoglycan synthesis. Usually, if penicillin causes toxic reactions in humans this has nothing to do with its role as an antibiotic, this is a different issue of allergic response.

An important point to note here is that antibiotics like penicillin do NOT attack peptidoglycan that is fully formed, penicillins and related antibiotics only attack peptidoglycan as it is being newly synthesized while a bacterium is reproducing. Bacteria "at rest" – metabolically inactive - and not in the process of binary fission, are not synthesizing new peptidoglycan and are invulnerable to penicillin *as long as* they do not undertake a cell division– this has serious implications for the manner in which penicillin must be used. It is essential, as I mention below, that ALL of an antibiotic prescription be taken, or resistant and/or inactive bacteria may survive and cause more severe infection. This is a danger if penicillin is stopped too soon, there may well still be bacteria around because they are inactive and thus not vulnerable to the penicillin taken so far. The penicillin taken so far in the course of treatment will NOT kill those inactive bacteria, you must take all the penicillin so that there is time for the inactive bacteria to become active again, try to divide – and then they are vulnerable to the penicillin and will be killed.

Examples**:**

Penicillins - fungal products, activity based on **β-lactam** ring, if that ring is broken they are inactivated, they are usually better against gram positives, there are semisynthetic variants. The basic structure can be modified to allow parenteral (not by mouth) or oral administration, or to cause the drug to accumulate rapidly in kidneys etc.

Cephalosporins. Fungal products, their activity is based on the **β-**lactam ring. Often used when a person is allergic to penicillin, they have few side effects but are more expensive than the penicillins, newer versions are poorly effective against gram negatives.

Carbapenems, bacterial products, often used in combination with other drugs that prevent their breakdown in kidneys, they have a very broad spectrum of activity, Primaxin is a prime example, it is a combination of the carbapenem compound called imipenem with cilastin, the latter is not an antibiotic, it functions to prevent degradation of the antibiotic in the kidneys. Bacitracin is an example of another antimicrobial agent that attacks cell

walls of bacteria, it is a toxic peptide produced by the bacterium *Bacillus licehniformis*, it is often included in skin treatment creams.

Vancomycin is another example of an antibiotic that attacks cell walls, it is a glycopeptide – it is a powerful but often toxic antimicrobial agent, sometimes used as the "last ditch" treatment in otherwise resistant infections of enteritis. Vancomycin is poorly effective against gram negatives.

Antimicrobial agents that damage the bacterial cell membrane.
"Membranes are membranes" But, bacterial cell membranes have a different composition to ours and some agents attack bacterial cell membranes but not those of eukaryotic cells. Polymixins and Tyrocidins are examples. The Polymixins are bacterial products and are good against gram negatives, they are used in creams, but can be toxic when taken internally. Tyrocidins are bacterial products and are used against some gram positive wound infections, but they are very toxic.

Antimicrobial agents that inhibit bacterial protein synthesis.
Ribosomes of bacteria are smaller and slightly structurally different than eukaryotic ribosomes. Tetracycline, streptomycin, chloramphenicol, and erythromycin antibiotics bind to and inhibit the activity of bacterial ribosomes in a number of ways, but do not affect eukaryotic ribosome function. Our *mitochondria* have "bacterial type" ribosomes, so prolonged use of agents such as tetracycline (as is found in acne treatment) might affect our cell functions, but there is no great evidence that this actually occurs.

Examples:

Streptomycin and related drugs (**aminoglycosides**). One of the first antimicrobial agents, it is a bacterial product. Not commonly used now, many bacteria are resistant. Can work well when used synergistically with **β-**lactam antimicrobial agents in treatment of wound and burn infections, can be kidney toxic. Streptomycin was the first agent to have any useful effect against the tuberculosis bacterium.

Tetracyclines. Bacterial products, they have the widest spectrum of all antibacterial agents, good at killing bacteria inside our cells. Have to be careful since they can cause gut upsets, can be liver and kidney toxic. Can cause teeth mottling when used by the very young. Are inactivated if taken with calcium - as in milk or milk products.

Chloramphenicol. Bacterial origin, but now fully synthetic, can cause a rare but fatal anemia, use is now reserved for severe resistant infections.

Macrolides, such as erythromycin, bacterial product - bacteriostatic, low toxicity and used in combination with other antimicrobial agents.

Antimicrobial agents that inhibit bacterial nucleic acid synthesis.
Bacterial DNA replication is much the same as ours (being a bit simplistic), but the enzymes involved are structurally different. Agents such as rifamycin bind to bacterial DNA replication enzymes and inhibit them but do not affect ours.

Examples:

Rifampin (one of the rifamycins produced by *Streptomyces* bacteria). Blocks transcription (reading of the code) of RNA. A key drug in the treatment of tuberculosis. Can be liver toxic and can interfere with activity of other medicines.

Quinolones - nalidixic acid is the commonly used example, blocks unwinding of DNA and thus interferes with transcription and/or cell division.

Antimicrobial agents that act as *anti-metabolites.*
The complex metabolic events in a cell are undertaken by enzymes. Each enzyme acts only on a very specific molecule - its **substrate**. Anti-metabolite type antibiotics "look like" one of these natural substrate molecules (the correct term is that they are **structural analogues** of substrates) but are NOT the same and they interfere with the activity of the enzyme in question because the enzyme "preoccupies" itself with the anti-metabolite antibiotic instead of its proper substrate. If the activity of that enzyme is crucial then the cell will die or fail to grow and reproduce when the antimicrobial agent is taken, because the essential compound that the enzyme should be producing is not being produced. Sulfa drugs are a good example of antimicrobial agents that are anti-metabolites, they are similar to an essential metabolite needed to form folic acid in bacteria and interfere with or "occupy the attention" of the enzyme which performs that step in bacteria (but this enzyme step is not found in OUR cells, we get folic acid from our diet). Some anti-metabolite antibiotics are similar to the building blocks of DNA and interfere with that process (some anti-viral agents are in this category).

Examples:

Sulfonamides - the sulfa drugs, most ancient of all the antimicrobial agents still used in man, all are synthetic. They can cause allergic reactions and are toxic, though newer versions are less so, and are used in urinary tract infections. Sulfa drugs are often used in combination with other compounds such as trimethoprim which improve their effectiveness. Trimethoprim is not a sulfa drug, it is combined with the sulfa drug sulfamethoxazole where the trimethoprim has a synergistic effect since it inhibits a different step in the bacterial folic acid synthesis pathway, this trimethoprim-sulfamethoxazole combination is an important treatment of urinary tract infections, and is also used to treat *Pneumocystis* infections in AIDS patients, especially since it is very effective at penetrating the brain and cerebrospinal fluid, has a broad spectrum of activity and can be taken orally.

Isoniazid, an antimetabolite to the production by some bacteria of niacin and vitamin B6. Used to treat tuberculosis, where it is usually given in combination with other drugs.

Some side-effects of anti-microbial agents:

Toxicity – antimicrobial agents or their breakdown products can sometimes act as **toxins**, this can include liver and kidney damage (sometimes the liver converts harmless anti-microbial agents into toxic compounds).

Allergy. As is found with penicillin and sulfa drugs for instance. Can range from a relatively minor problem, to a life threatening case of **anaphylactic** shock.

Disruption of the normal gut flora. Long term use of oral antimicrobial agents can kill gut bacteria and disrupt normal gut function, causing bloating, diarrhea, distension and pain, a problem particularly with wide spectrum antimicrobial agents like tetracycline.

Resistance of microorganisms to anti-microbial agents.

One of the ways this can happen is by evasion of the **immune response**. **Tuberculosis** is a classic example, where the bacterium "walls itself off" in small **tubercles** so that the immune system has poor access to it. This walling off response also impedes access of antimicrobial agents to the bacterium

Of paramount concern though is genetically based acquisition of resistance to anti-microbial agents – **mutation** is the basis of this process. It is MOST important that you understand that a mutation which arises in a bacterium to render it resistant to an anti-microbial agent is RANDOM. This can be a difficult concept to grasp and so I will illustrate this more fully in class.

The genetic basis of resistance: This is resistance which is genetically encoded and passed on generation to generation: examples are-

-Alteration of targets. Mutation of DNA in a gene responsible for the synthesis of the bacterial ribosome may produce an altered ribosome that still functions normally, but has a subtle alteration in shape so that agents such as tetracycline or streptomycin can no longer bind to it and disrupt protein synthesis.

-Alteration of membrane permeability.
Mutations may occur which alter properties of the bacterial cell membrane so that a given antimicrobial agent (such as tetracycline for instance) can no longer get into the cell in order to act on its target.

-Development of or acquisition of enzymes. Sometimes bacteria acquire an enzyme which can inactivate an antimicrobial agent, such as penicillinases, properly called **β**-lactamases, these destroy the active structure of penicillin type antimicrobial agent which possess a critical **β** -lactam ring. Very often the genes for these antimicrobial agent inactivating enzymes are transferred amongst bacteria - I will demonstrate how this is done in class.

-Alteration of enzymes. Sometimes a subtle change is produced in the structure of an enzyme produced by the pathogen (by mutation) which can cause an antimetabolite (a sulfa drug for instance) to be unable to bind with the enzyme and inactivate it, the enzyme continues to function (the enzyme no longer "recognizes" the antimetabolite).

-Alteration of a metabolic pathway. Sometimes there is a mutation in a bacterium so that the step that would be inhibited by an antimetabolite is no longer functional, yet the product of the pathway is still formed, because an alternative pathway becomes operative. Or perhaps the bacterium becomes able to obtain that particular product from its environment instead of having to make it, so that the effect of the antimicrobial agent becomes irrelevant.

There are a number of ways to limit the appearance of drug resistant bacteria.

1) Do not take bacterial antimicrobial agents unless you need them. This a societal responsibility, you have the responsibility, for instance, not to pressure a physician for antimicrobial agents when you are suffering from a cold without any bacterial infection complications. Unrestricted irresponsible use of antimicrobial agents increases resistance problems. Physicians must also resist pressures to prescribe such antimicrobial agents when they are not needed.

2) When you are taking antibacterial antimicrobial agents appropriately, take ALL of them - finish the course. If you do not, you are in danger of selecting for the most dangerous of bacteria and then unleashing them on the rest of us. This kind of misuse of antimicrobial agents can result in you becoming an asymptomatic carrier of infectious bacteria that no longer harm you, but can make others sick.

3) Use antimicrobial agents only for their intended purpose, and not as an inclusion in animal feed just to increase weight gain.

There are some ways in which drug resistance can be limited, one of these is to use a **synergistic** effect (an additive effect where two agents have a greater combined effect than when used separately) the commonest example of this is the use of **clavulanic acid** in combination with amoxicillin (a penicillin type antimicrobial agent). Clavulanic acid is not an antimicrobial agent, it an inhibitor of the beta lactamase enzymes carried by many bacteria that break down and deactivate penicillin type antimicrobial agents like amoxicillin. Another example is the use of streptomycin in combination with penicillin, the penicillin damages the cell wall in a way that allows the streptomycin to enter the cell more efficiently, where it exerts its effect. The converse of this has to be taken into account too, the **antagonistic** effect, where one agent acts against the effectiveness of another, using penicillin and tetracycline together can result in this, the tetracycline stops growth of the bacterium, and it if is not growing, it is not forming new peptidoglycan and thus is not vulnerable to the penicillin.

How do we figure out the best antimicrobial agent to use?

Just a quick look at this using the **Kirby Baue**r test, I will illustrate this further in class or tutorial. When a paper disc containing an antimicrobial agent is placed on to a "lawn" of test bacteria on agar, a "zone of inhibition" will appear around the disc, a ring of clear agar where the bacteria did not grow because of the action of the antimicrobial agent diffusing out from the disc, in general, the greater the zone of inhibition, the better the antimicrobial agent may be as a choice to treat an infection involving the test bacterium on the agar.

In addition, once a physician has identified the species of bacterium that is causing an infection it is often not necessary to do a test to find the appropriate antimicrobial agent, there are a wide range of recorded data that can be used to select an antimicrobial agent.

Anti-fungal agents:

Since fungi are eukaryotes, the antimicrobial agents used to treat them tend to be more frequently toxic than antibacterial agents, and avoiding this toxicity by giving lower doses means it can take a long time to effect a cure. It is also more difficult to conduct lab tests to optimize treatment with antifungal agents.

Imidazoles and **triazoles**. Disrupt fungal cell membranes by interfering with sterol synthesis. Used in creams against surface rashes due to fungi (dermatomycoses).

Polyenes; **amphotericin B** - perhaps the most commonly used antifungal agent for internal (systemic) infections although there are numerous side effects, especially kidney toxicity. **Nystatin** is used in the same manner as amphotericin B, but it is better than that agent when used to treat intestinal fungal infections.

Griseofulvin, mostly used for skin infections by fungi, but also can be used internally.

Tolnaftate, flucytosine, terbinafine - newer drugs used against problems such as athletes foot and jock itch.

Antiviral agents:

Many of these are structural analogues of nucleotides – the building blocks of DNA. They incorporate into replicating DNA chains during viral gene replication and cause their synthesis to be disrupted and terminated. We will not discuss them in any detail except to point out that agents such as **AZT** (azidothymidine) which are used to treat AIDS are nucleotide analogues.

Amantadine can be used to treat established influenza, but it is dangerous and is thus only given where the benefits outweigh the risks very clearly, as in the very aged with poor immune function. There are now numerous reports of resistance.

Newer antiviral drugs such as tamiflu® act to prevent penetration of viruses into cells.

Interferons are natural animal cell products that function in a form of defence against viral infection. An infected cell produces interferons and similar factors, which induce neighbouring cells to release proteins that prevent them from being infected.

Anti-protozoan agents:

Quinine. One of the earliest agents against malaria, found in the bark of the cinchona tree, now mostly replaced by synthetic versions **chloroquine** and **primaquine**. Quinine and its variants can be toxic and can cause hearing damage and there are resistance problems. **Artemisin** is an anti-malarial drug developed from ancient practices in China, it is effective, and it is extracted from a plant.
Metronidazole (Flagyl), used to treat *Trichomonas* vaginal discharge and also *Giardia* ("beaver fever"), since it acts against DNA it may cause birth defects and cancer.

Parasitic animals

There are a number of agents against helminths ("worms"), **piperazine** and **ivermectin** are good examples which are used against roundworms and nematodes.

10. Disease.

A microbe may be in one of a number of **symbiotic** relationships with its host. Symbiosis is a relationship between two or more different species:

In a **mutualistic** relationship both partners benefit, as is thought to be the case with certain of our gut bacteria which receive a safe warm nutrient rich habitat and in return synthesize vitamin K and some B vitamins that we can absorb and use, or with the gut bacteria of termites that receive a safe environment in return for the products of the decomposition of cellulose that they carry out.
In a **commensal** relationship neither host nor microbe are harmed, as with our microbial skin inhabitants.
In a **parasitic** relationship one partner benefits, the other is harmed, this is the situation that concerns us - microbe based disease.

A microbe that has the capacity to cause disease in a host is called a **pathogen**, and will produce varying intensities of disease depending on its **virulence** – the degree of aggressiveness with which it can cause disease. The degree of virulence varies amongst microbial species, some cause only mild problems such as a weak enteritis, others, such as the rabies virus are highly virulent, once infected the result is virtually always fatal if action is not taken very quickly with a vaccine. Even strains of the same pathogen, the same species, can vary in virulence, this is seen with *E. coli* for instance, where many species in the gut cause no problems, but some, such as those that carry specific genes for toxins, can cause very serious disease. Serial passage of pathogens through animals of the same species can sometimes increase their virulence. However, in other situations, such as when using certain laboratory techniques, the virulence can be **attenuated**, which means to lessen it. This can be done by **transposal** for instance, where the pathogen is

transmitted through a different animal host many times, this can weaken it, and the weakened form can be used in a vaccine. Pasteur used this method to attenuate rabies virus so that is could be used as a vaccine.

We have within us and on us a range of normal **microflora** - microbes usually present and not causing active disease, some authorities prefer the term microbiota rather than microflora, the term flora is historical, it came to be used in the early days when many microbes were believed to be plant related, but I will stick to using the older term. It is possible for normal resident microflora (such as on the skin, in the nose, vagina, or gut) to cause disease if there are changes such as a disruption of the numbers and types of microflora because of factors such as antimicrobial agent treatment, stress, other diseases such as cancer, immune system defects etc. Some normal microflora are **opportunistic** pathogens, they do not cause disease when they are present in a normal healthy person, often because the dynamic balance of a complex microbial community - the normal competition amongst these microbes for nutrients and survival (often called **microbial antagonism**) prevents them from being aggressive pathogens, but in disturbed circumstances such as destruction of other competing bacteria, a compromised immune system, their introduction into the body through a wound etc, they may then cause disease.

Koch's **postulates** are used to verify that a given microbe is truly responsible for a given disease:
-Must find the organisms in every case of the disease.
-The organisms must be isolated from the diseased animal and grown in pure culture.
-The disease must be caused by pure cultures of the organism when it is inoculated into healthy hosts.
-Must re-isolate that organism from the inoculated diseased host.

It is not always possible to satisfy or perform Koch's postulates.

Of course, not all diseases are of infectious origin. Also, note two terms; **iatrogenic** diseases are caused by medical procedures (often it is infectious disease). **Idiopathic** diseases have unknown causes.

Communicable diseases are easily spread from one person to another by touch or by sneezing etc, such as flu. **Non communicable** diseases may be caused by an infectious agent, but are not easily or usually transmitted by touch or sneezing, some food poisonings are an example, or a wound infection such as tetanus.

Contamination is the appearance of potential pathogens on or in you but it does not necessarily imply infection, dirty hands are contaminated by microbes, but you can avoid infection by washing them.

An **infection** is a multiplication of pathogens in or on a host, though sometimes the word **infestation** is used when the organism is a larger parasite such as a worm or lice. Infection begins with a **primary infection** in which a bacterium or virus or parasite

initiates an infectious disease, and sometimes this ends up with a **secondary infection** where other microorganisms "take advantage" of opened wounds and lesions and perhaps a weakened general state, to initiate a further infection. In some cases the secondary infection may be a **superinfection** – a highly virulent rapidly progressing and dangerous infection. A **focal** infection is **local**, and from such a site (a boil for instance) bacteria and toxins may spread, abscessed teeth are focal infections. Some infections are **inapparent**, the sufferers are carriers, they do not feel unwell and show no signs of disease, but they spread the disease, this is found with hepatitis B for instance.

Epidemiology is the study of the factors and processes of disease, and how diseases spread. Epidemiology also assesses the causes (**etiology** is the technical epidemiological term for the **cause** of a disease) of disease. A disease may just be **sporadic** - a low level of local and random cases that are not of public health concern. If a disease is **endemic**, it is present in a population at constant lower levels that are not of public health concern. **Epidemics** are sudden high numbers of a disease in a population that are a public health concern. **Pandemics** are serious worldwide outbreaks of disease.

There may be **reservoirs** of pathogenic organisms, a reservoir is a site where microorganisms can persist and thus maintain the ability to cause infection. Some humans for instance are reservoirs and **carriers** (definition: carry the infectious agent but do not exhibit the disease themselves) of *Staphyloccocci* which do not cause disease in them but can cause food poisoning in non carriers, this can be a problem if unidentified carriers are working in the food preparation industry. People may also be reservoirs of *Pertussis* bacteria which can cause whooping cough in others. Such human carriers may shed the bacteria by touch, aerosol (sneezing) or in feces for instance.

Other animals may act as reservoirs for pathogens that attack humans (diseases caused by these pathogens are called **zoonoses**), there are many, such as rabies. Water and soil may harbour these pathogens.

Pathogens may enter humans through orifices such as mouth, nose, genital openings, wounds in the skin, ears, eyes.

Vectors may spread disease. These are living organisms that act as "intermediates" in the transmission of a disease, with bubonic plague being a classic example. This is caused by a bacterium, but is transmitted from an infected rat to a human by a flea, which has fed on blood from the rat.

Disease can be very severe when it is transmitted to a population which lacks **herd immunity**, as was found centuries ago with North American native peoples who lacked herd immunity to smallpox or measles introduced by immigrant Europeans. In herd immunity perhaps 60-90% of the population is immune while 10% are susceptible, this makes it much harder for a disease to spread.

Control of a disease – CDC –Centre for Disease Control (based in Atlanta Georgia), the premier world disease analysis agency, they conduct immunization campaigns,

they supervise the isolation, identification, quarantine, and control of causative pathogens and of vectors. The CDC publishes a weekly Morbidity and Mortality report that tracks new diseases, outbreaks, and epidemics. You should distinguish between these two terms because they are relevant to your understanding of the epidemiology of disease, **morbidity** refers to the relative numbers of individuals affected by a given disease in a set period of time in a given population, it is NOT, as is often the case when this word is used in common conversation a measure of the number who die of a disease, the word **mortality** refers to the number of deaths.

Nosocomial infections are those that one gets (contracts) in a hospital doctors office, clinic etc. We will discuss this in class briefly, but note two points here for that discussion, many people in a hospital are very ill and compromised, and a hospital is a prime place to find a "superbug".

Factors which enhance the ability of a microbe to cause disease - which aid pathogenicity (Virulence factors);

I mentioned elsewhere the notion of virulence – the degree to which a pathogen can cause a disease – a measure perhaps of the "aggressiveness" of the pathogen. Sometimes a bacterium has to be present in high numbers in order to establish infection, sometimes just a few numbers are needed. This is often expressed as the $\mathbf{ID_{50}}$ which is the infectious number that will cause disease in 50% of the population. The portal of entry affects the $ID_{50,}$ anthrax can be caused by 10-50 endospores if they enter through the skin, but 10,000 to 20,000 are needed to cause infection if they are inhaled. For the Walkerton strain of *E. coli* the ID_{50} is extremely small, this is a highly virulent strain and only a few bacteria are needed to initiate disease.

Adherence factors can be special proteins - **adhesins**- that allow a bacterium to attach to host cell membranes, or they can be a slimy **capsule** that allows bacteria to stick to the teeth, or **pili** which allow some bacteria to adhere to the inner wall of the bladder and genital tract.

Some pathogens are more **invasive** because they produce virulence factors like **hyaluronidase** - an enzyme that digests the material that attaches host cells to each other and this allows the bacteria to pass between cell layers, or **coagulase** which allows formation of blood clots that encapsulate bacteria so that they can be protected from the immune system and can grow, and **streptokinase** which can subsequently break up those blood clots to allow the bacteria to spread further.

Many bacteria produce toxins. **Exotoxins** are a range of molecules actively synthesized and released by living bacteria (mostly gram positive) into hosts, which have a wide range of effects, some are nerve toxins, some destroy red blood cells or hemoglobin, some damage liver or kidneys or other organs. **Endotoxins** (LipoPolySaccharides - LPS) are part of the structure of the outer membrane of gram negative bacteria and can cause catastrophic shock reactions, especially in contaminated systems used with kidney dialysis patients. LPS toxins are generally only released by dead gram negative bacteria.

LPS toxin can be a concern when large numbers of infecting gram negative bacteria are killed during antimicrobial agent treatments, this can suddenly release enough LPS into the circulation of patient to cause a shock reaction.

Some virulence factors are enzymes such as **hemolysins**, commonly produced by *Streptococcus* species for example, they burst (lyse) red blood cells. The coagulase, streptokinase and hyaluronidase mentioned above are virulence factors and are also enzymes.

Very often a virus causes its disease symptoms by directly killing cells, this is the **Cytopathic effect** (CPE) a term specifically applied to viruses, or sometimes a virus does not immediately kill cells but causes infected cells to release many copies of the virus. In many cases the unpleasant symptoms of a virus infection are more a result of actions of the immune system - generally a result of release of histamines which promote tissue swelling, fever etc. Remember, sometimes viruses infect but do not cause disease until months or years later – **lysogeny** or **latent** infections.

Fungi can infect in similar ways to bacteria, and like bacteria, can produce a wide range of toxins which have varied effects and cellular targets. Fungi also frequently cause allergic reactions, especially by inhalation of spores.

The definition of a **sign**, a **symptom** and a **syndrome;**
Sign – characteristics of a disease that can be observed by somebody else.
Symptom – a characteristic of a disease only observed or felt by the patient.
Syndrome – combination of signs and symptoms that occur together and are indicative of a particular disease.

Some infections are **acute**, with rapid onset of symptoms (like flu), and some are **chronic** diseases - like leprosy or tuberculosis. Some infections are **local** (a boil, abscess) and some may be **systemic** (throughout the body). In **septicemia** bacteria are present and they proliferate (grow) in the blood, in **bacteremia** and **viremia** the bacteria and viruses are present, respectively in the blood, and are transported by the blood but do not multiply in it. In some cases exotoxins generated by bacteria spread from the local site of infection through the blood - this is called **toxemia.**

In a typical course of an infection there will be a short phase where the microbe incubates and grows in numbers but no ill effect is noted - this is the **incubation phase**, then a **prodromal** phase follows where symptoms are first noted, involving perhaps mild unwellness, then the **invasive** phase where the infection is established and the full signs and symptoms of the disease are found - such as fever, rash, and chills or swelling, headache etc, the infection reaches its greatest intensity in this phase (what is called its **acme**), and if the invasive phase appears very suddenly and is severe the invasive phase is described as **fulminating**. This invasive phase is followed (if you are lucky!) by a

phase in which the disease declines in severity as the immune system "gets the upper hand" – the **decline** phase, followed by **convalescence**,

Note the caveats that will make it difficult to eradicate infectious diseases:
Parents fail to get children vaccinated.
Infectious agents are highly adaptable.
Human activities can cause previously unknown or rare diseases to become a problem.
Problems are increased by immigration, commerce, travel.

11. The immune system, a brief description.

Immunology is a complex topic. I have given a short version below.

You have what can be described as **genetic immunity**, as an example, some viruses attack dogs but not you, your cells do not have the same cell membrane molecules as a dogs, and this means that the dog virus cannot recognize your cells and so does not infect them, this is a genetic difference. This genetic immunity (also called **innate** immunity) is NOT a consequence of the action of the **immune *system*** that we are going to discuss below.

There are **preliminary barriers** to foreign materials, such as the skin, the flushing effects of mucus on internal surfaces, competitive surface bacteria which ward off bacteria, antibacterial substances in tears and saliva, and so on, but these are not components of the immune system proper.

Some scientists take the approach that in many animals there is what is called an innate immune response, which consists of non specific generalized defence responses against infectious agents but that this evolutionarily older response is not an "immune response" proper, and that the "true" specific immune response, involves recognition of a specific microbe or virus and a targeted response, by special cells (T cells and B cells, macrophage etc). The belief is that this true immune system is a more evolutionarily recent system first found, by many accounts, in cartilaginous fishes such as ancient sharks.

I take the approach that there is **an immune system** that consists of two components, the first component is the **non specific** immune response – composed of the inflammatory response, interferons and the complement system – a general response not (at least initially) recognizing the specific antigenic nature of an invading microbe or virus. The second component, usually cooperating with and at times controlling the non specific component is the **specific** immune response, triggered by the specific recognition of microbial or viral antigens, and it is undertaken by B cells, T cells, macrophage etc.

So, the human body has an **immune system** the task of which is to distinguish **self from non-self** and to attack and neutralize foreign (non self) matter in the body, whether it is molecules or cells. A well established and verified theory in immunology, the **clonal selection theory**, states that the body is able to distinguish self from non-self and in

response it triggers specific cells that have specifically recognized a substance as being non-self, to rapidly multiply their numbers (to form clones of themselves) which will then "fight" the non-self substance. Also, once a body cell transforms to become a cancer cell it is now "non self" and is (usually) promptly recognized as such and destroyed by the immune system.
The immune system consists of the action of special cells, and the chemicals they produce, these cells are largely produced in the bone marrow. "White cells" in the blood are the specific immune system cells.

Inflammation is a non specific immune response, part of this process is **tissue swelling** as might occur following a local infection by a bacterium. The bacteria killing cells of the immune system (such as **macrophage** and **neutrophils** which ingest bacteria, and also **B cells** which release bacteria neutralizing antibodies) are in the blood and the lymph. Normally, cells in the blood do not pass through capillaries into body fluid, but this HAS to occur if bacteria fighting cells normally resident in the blood are to attack bacteria outside the blood circulation – in the tissues that have been injured or invaded. Special chemicals (**histamines**) make capillaries leaky to cells and some proteins and fluids (a process called **diapedesis**) so that infection fighting immune system cells can get to the infection site, and the region where this happens becomes swollen with fluid (this is called **edema**). This edema causes the site of infection to be tight and painful, something you will recognize from when you have had minor infections. The histamines are released from **mast** cells found in the walls of capillaries, they cause small local blood vessels to swell and this gives the redness around the inflamed swelling of the infected area, which also feels hot. Because the area is now swollen and under pressure it presses on pain sensors, you sense this pain, you try to keep the inflamed area still and this hastens recovery.

A generalized feeling of hotness during an infection, what we call **fever**, is often caused by small molecules called **pyrogens**, of many different kinds, produced by microbes themselves, these are exogenous pyrogens, and they trigger immune system cells, especially macrophage, to release endogenous pyrogens, which then travel to the brain and induce it to trigger a series of actions that raise body temperature to cause a fever. A fever can actually help to fight an infection, since some bacteria do not multiply well at higher body temperatures, and the immune system may act faster when the body temperature is higher.
Macrophage (white cells that look like and act like amoeba) in the infection area ingest bacteria and release chemical signals called **interleukins** which attract more white cells (B and T cells), these pack the local infected area as they fight bacteria and all these cells and damaged tissues form the **pus** we see at such sites, pus includes dead pathogenic cells, injured and dead host cells such as phagocytes (macrophage etc) and debris, as well as active white cells.

Another non specific component of the immune system that participates in the inflammation process, is the **complement protein** system. When directed against a bacterial cell, because an **antibody** is already bound to it, or because the first complement

protein in the binding sequence recognizes a specific sugar on the bacterial cell surface for instance, a number of special complement proteins (normally present in the blood) bind to the bacterial membrane in a well defined order, and at a certain point this process becomes "automatic" - the assembling proteins will form many **pores** in the bacterial cell wall which will cause it to burst (lyse) and leak out all its contents and die. Once the complement system has begun to form on the cell surface and this process goes beyond a certain point, it is uniformly fatal, it cannot be stopped. Complement proteins can also increase the inflammatory response.
Granulocyte cells such as neutrophils, eosinophils, basophils either "eat" -phagocytose bacteria or release enzymes and chemicals which neutralize bacteria or parasitic organisms such as microscopic "worms".

The **specific component** of the immune system consists of the synchronized action of **T cells and B cells** in combination with components of the non-specific immune system which are controlled by the specific component. T cells (also called T lymphocytes) are produced in the bone marrow and undergo a maturation process in early fetal life which is controlled by the **thymus** gland (the T in T cell stands for thymus). For the rest of this discussion, for simplicities sake, I will call the specific immune system just the "immune system".
The immune system has **specificity**, any given B or T cell (and there are billions of them) only recognizes one very specific molecular shape on what is recognized as a foreign object, this shape is called an **antigen**. So, any molecular structure recognized as foreign by the immune system is called an antigen. Recognition of this antigen by the immune system prompts the **immune response**. Those B and T cells, and only those cells, that have recognized the particular foreign antigens will then reproduce rapidly and those new cells in turn reproduce and so on, amplifying and concentrating the immune response, this is the clonal selection response mentioned earlier. This is the response mounted on first "seeing" the antigen, and it is called the **primary** immune response. If you survive the infection, a number of these T and B cells which undertook the primary response, become **memory** (**anamnestic**) cells, T and B cells of the specific immune system that can survive for years, and if later on, they encounter a second assault by the antigen they specifically recognize (on a virus for instance) they mount a much faster **secondary** response.

The secondary response is generally so fast that you do not sense any problem - you had acquired **immunity**. Thus, an essential feature of the immune system is its **specificity**, it only recognizes (usually) specific foreign antigens and a response "tailored" by the immune system to those antigens will not work against other antigens. Implicit in this notion of specificity is that the immune system does not attack you, it has acquired what is called immune **tolerance** for your cells and molecules, this occurs early on in fetal development and particularly in the thymus gland, where any T cell of the immune system that has the undesirable potential to react against your cells, is destroyed. This tolerance formation is not perfect though, we can suffer from **autoimmune** diseases such as rheumatoid arthritis or multiple sclerosis where your immune system does attack your own cells. **Allergies** are inappropriate responses by the immune system to *harmless*

substances.

There are several kinds of T cell. **T helper** cells are the "Major Generals" of the specific immune response, they **control** much of it, they release factors which marshal other cells and components into the local sites of the immune response. T **cytotoxic** cells can kill foreign cells or our own infected cells by direct contact. There may also be T **suppressor** cells which shut down the immune response, but there is now controversy about this, it is thought there are suppressive cells in the immune system but maybe they are not T cells.

Often the first event in recognizing a foreign invader is performed by a **macrophage** (a cell with amoeba like properties), which ingests the invader (a bacterium or virus for instance) breaks it down, and then expresses molecules (antigens) taken from the invading cell or virus on the outer surface of the macrophage in combination with special immune system recognition receptors on the macrophage membrane surface called **MHC** receptors. These combinations are the **MHC-antigen complex.** These MHC-antigen complexes are what the T cells recognize and bind to, and then the immune response begins, the bound T cells then recruit other immune system cells to the area and they also begin to divide and increase in numbers.

The T cell which has bound to the MHC-antigen complex on the macrophage sends out a chemical signal which activates B cells that also specifically recognize this antigen, to cause the B cells to mature and excrete many **antibody** molecules which bind with and neutralize the invading cells not yet ingested by bacteria, this "coating" with antibodies often directly inactivates invading cells, but the antibodies now bound to the invading cells or viruses also act to signal the immune system to the presence of the invaders and thus intensify the immune response further. B cells which are activated and producing antibodies are called **plasma** cells. B cells can also ingest antigens that they specifically recognize and present them on their own membrane surfaces in combination with their MHC molecules, when this occurs a T cell can bind with and recognize this antigen-MHC combination and this activates the T cell to release small chemical messengers called interleukins that are involved in coordination of the immune response.

My personal explanation for this complex series of events involved in the initiation of a specific immune response is that it prevents or minimizes inappropriate immune responses, which can be dangerous and even fatal. In principle, the immune system has autonomy, there is no signal from the brain that activates it. You can think of the immune system as a "pitbull on a leash" , and you do not want to let it loose unless it is under firm control. When a foreign infectious bacterium is in the bloodstream for instance, a macrophage phagocytoses it, breaks it apart, and then displays molecule size fragments of the bacterium on its own membrane surface – but, it does so in association with the macrophage cells <u>own</u> recognition molecules, it is as if the macrophage – is "saying" "hey you guys in the immune system, this bacterium I have ingested is a serious threat, and I have displayed it on my own surface with my own recognition molecules as a specific indication of this fact, it is time for you to act". The MHC molecular complexes that are on all of your cells are the molecules that your immune system uses to recognize

YOU. What this complex series of steps does is to *present* the foreign material *to* the immune system in a manner that allows control of the response in a coordinated series of actions. Anyone who suffers from a serious autoimmune disease like multiple sclerosis can tell you how bad it is for the immune to "go off on its own" and initiate uncontrolled and inappropriate immune responses.

There are five types of B cells with respect to the type of antibody they produce (antibodies are collectively called **immunoglobulins**), **IgD, IgM, IgG, IgA, IgE:**

IgD is present in fetal stages of immune system development and plays a poorly understood role, if any, in the mature immune response. IgM is the antibody produced in the primary immune response, it is a huge molecule and is actually composed of 5 antibody molecules linked together wagon wheel fashion. IgG is the antibody most commonly involved in the secondary immune response, these antibodies are long lasting, can cross the placenta and are present in mothers milk. IgA antibodies are involved in immune responses to infective agents on mucus membrane surfaces, when they are secreted onto membrane surfaces they are bonded together into pairs and in this state IgA is referred to as secretory IgA. IgE antibodies bind to mast cells causing histamine release, especially during inflammatory responses to parasitic infections. IgE figures prominently in potentially fatal **anaphylactic** allergic reactions.

Vaccination is "borrowed immunity", as an example, a harmless extract of a pathogen like the diphtheria bacterium is prepared, maybe it is whole but killed cells, or it is weakened live cells, or maybe it is bits of the bacterial cell wall, but the extract itself will not cause disease because no virulent bacteria are present. When the extract is **inoculated** into you (the process of vaccination) it will trigger a primary immune response without an infection being caused, memory cells will be formed, and on subsequent exposure to real, live diphtheria causing bacteria, a powerful secondary immune response will occur and no infection will be established. You have obtained **immunity**. In some cases immunity is life long, in other cases, it is shorter lived and repeat immunization is required.

In other cases, perhaps if one has been exposed to the tetanus bacterium and it has produced toxin, **passive immunization** is performed, antibodies against the toxin are inoculated into the bloodstream of the person to inactivate the toxin. In this case another animal (like a horse) was inoculated with inactivated toxin (inactivated toxins are called **toxoids**, they cannot cause harm because they have been subtly altered by heat or chemicals) and it developed immunity and its antibodies (**immune serum globulin**) were harvested. In the patients body these antibodies bind to and inactivate the toxin and protect the patient.

12. Infectious Diseases of the skin and eyes, wounds and bites.

Since the **skin** is the largest organ of the body and has a large surface area it acts as a fundamental barrier to infectious agents, it is a complex collection of tissues containing a waterproofing protein - **keratin** and is constantly renewing itself.

Skin secretes substances which deter microbial invasion such as oils and sweat.

One can think also of the mucous membranes which line the inner surfaces of the body, as being an "internal skin". These surfaces often have specific transport functions and are moist, and also release mucus which protects against microbes, along with a specific antibody molecule (immunoglobulin) called **secretory IgA** which can intercept some microbial invaders and defend against them.

There are a huge number of microbes of many species which constitute the normal microflora of the skin, the majority are bacteria, but fungi are also found, such as the ubiquitous yeast *Candida*. Moist areas of the skin, in folds and in armpits and pubic regions will naturally harbour more bacteria and fungi. The majority of the bacteria that are normally found on human skin are gram positive.

Skin acts as a specific and non specific site for defence against infection.

Examples of bacterial skin diseases:

Staphylococcus.

Gram positive coccus, forms clusters of cells (the prefix staphylo- literally refers to a bunch of grapes).
Facultatively anaerobic.
Occurs on skin and nasal passages of healthy humans and animals.
Most "staph" diseases are caused by *S. aureus*.
50% of *S. aureus* strains produce a heat stable **enterotoxin** when growing in contaminated food – this cannot be destroyed by usual food heating procedures.
S. aureus also causes **pyogenic** (pus forming) infections such as impetigo, boils, abcesses, pneumonia.
Pathogenic staph also produce and excrete pathogenesis enhancing proteins such as coagulase, leukocidin, hemolysins.

Staphylococcal infections; furuncles (boils), pustules, carbuncles. Easily spread by contact or aerosol from unsuspecting asymptomatic carriers.

Scalded skin syndrome, a *Staphylococcus* infection resulting in skin which looks as if it has been burned with boiling water - a result of particular **exfoliating toxins**, most often found in infants.

MRSA (methicillin resistant *Staphylococcus aureus*) often pronounced as "Mersa" is becoming a serious issue as evidenced by recent news stories, these staph bacteria are

resistant to the usual penicillin type antibiotics used for staph infection (such as methicillin which is a penicillin type molecule). MRSA bacteria are generally spread by asymptomatic carriers especially in situation where skin can become abraded, as in sports like football and hockey, and can be a serious issue in hospitals and other health care facilities. These are dangerous infections in some cases and have caused fatalities, but most such infections can be treated with more powerful antibiotics such as Vancomycin, and can be minimized by appropriate avoidance procedures.

Streptococcus.

Gram positive coccus, forms chains of cells.

Facultatively anaerobic.

S. pyogenes and *S. pneumoniae* (pneumococcus) cause most of the human "strep" diseases.
Pneumococcus is generally present in normal healthy people.
S. pyogenes is rarely present in normal healthy people.
pneumococcus causes pneumonia, otitis media, meningitis.
S. pyogenes causes impetigo and "strep throat".
S. pyogenes can produce cytotoxins, hemolysins, coagulases erythrogenic toxin.
Sometimes a Strep infection can result in rheumatic fever that can in turn permanently damage the heart.

Streptococcal infections: **Scarlet fever** (*S. pyogenes*) - these bacteria have a phage (a bacterial virus) which carries red rash producing toxin, can also be responsible for "strep throat". Can cause internal complications such as rheumatic fever, heart damage, a kidney damaging reaction called **glomerulonephritis**.

Erysipelas. St Anthony's fire, at one time a killer infection of small wounds and abrasions, not too common now.

"Flesh eating disease" (necrotizing fasciitis) is caused by certain strains of *Streptococcus.*

Pyoderma and impetigo, staph and strep or other bacteria may cause this pus forming highly contagious infection, usually easy to treat if caught early - with penicillin.

Acne - generally a pubertal chronic **pustule** forming infection though it may be more deep seated (cystic acne), caused in some fashion by interaction of normal skin bacteria with an excessive production of oils. Treated with topical creams, vitamin A in cream (acid) and tablet form, long term antimicrobial agent s etc. Involves a number of bacterial species including *Streptomyces and Propionibacterium*.

There was a CBC news story on Nov 8 2007, regarding drug resistant *S. pneumoniae* which had caused meningitis in a baby (and similar problems in other infants have been reported recently) in Toronto, physicians have to use adult drugs to treat babies infected

with this strain (19A), and the prevalent vaccine given to babies against *S. pneumoniae* (Wyeth's Prevnar) does not protect against this strain as of November 2007.

Examples of Viral diseases of the skin.

Rubella - German measles, causes a skin rash, generally mild but can have severe consequences to a pregnant female - can cause birth defects. Preventable by vaccination.

Measles - rubeola. Fever and rash causing, prevented by vaccination. May rarely have an encephalitis complication, one form of which is extremely rare and always fatal.

Chickenpox. The same virus (a Herpes virus) causes chickenpox as an immediate consequence of infection and then resides dormant in nerve endings only to reactivate in some people years later to cause painful nerve lesions called **shingles**. Vaccination protects. Must be careful when giving aspirin to children who have chickenpox, a few may develop **Reyes syndrome** as a result - which can be fatal.

Smallpox has been eradicated as a worldwide disease, though it remains stored in some laboratories. My generation was the last to undergo the effective vaccinations required to protect against the disease.

Fungal infections of the skin. Already touched on earlier, refer to earlier notes.

Microbial diseases of the eye;

Bacterial - *Chlamydial* and **gonorrheal** infections caught during childbirth, conjunctivitis - often called **pinkeye.**

Trachoma - a very common cause of blindness causes by *Chlamydia trachomatis*, especially in Asia, South America and Africa.

Viruses cause eye diseases - various kinds of conjunctivitis.

Some **parasites** cause eye disease, most especially a very nasty one called **Onchocerciasis** - also called river blindness in Africa and Central America, and a small worm that causes loa loa.

Wound and bite infections - not covered in detail here, but there are some important ones to mention.

Of course, there are a lot of types of bacteria that can get into a wound or bite - depending on where the wound occurs, and what causes the wound, and the type of infection depends especially on the mouth microflora of the animal where a bite is concerned - most of these respond quickly to antimicrobial agent treatment.

Clostridia, some of these bacteria are serious wound pathogens.

Clostridia are Gram positive rods, obligate anaerobes, Endospore formers

Cause botulism = *C. botulinum*

Cause gangrene = *C. perfringens* (also causes food poisonings)

Cause tetanus = *C. tetani*

Clostridia species produce powerful exotoxins.

Gas Gangrene is caused by *Clostridium perfringens* or similar *Clostridia*. Deep wounds (and sometimes diabetic ulcers) are contaminated with the bacterium where oxygen is low because it has been depleted in the infected tissue by other bacteria that use the oxygen, this allows the Clostridial endospores to germinate and the bacteria to grow.

The bacteria release toxins and enzymes that further destroy tissue producing deep dead (necrotized) wounds which blacken - this will spread and be fatal if not treated - sometimes requiring amputation. Lots of gas and foul smells are produced.

Cat scratch fever is caused by several types of bacteria, rat bite fever is also mainly bacterial.

Small animals like ticks cause skin infections, some of which are paralyzing, and some of these ticks spread bacterial infections such as the one that causes **Lyme disease**. Flea bites can spread disease as we have discussed previously