A framework for describing infectious diseases

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Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system. Although the cause of MS is unknown, we do know that CNS inflammation in MS is associated with demyelination and axonal injury, and it is conceivable that these effects are the result of an infectious agent. The damage seen in MS may be due to direct infection of the CNS or an immune reaction that subsequently leads to attacks on the CNS.

The purpose of this document is to provide an overview of the pathogenic mechanisms through which infectious agents (bacteria, viruses, protozoa, etc.) cause disease in humans. Using this framework, we can analyze the research conducted to date exploring the possible role of various pathogens in the development of MS, with the goals of identifying those pathogenic mechanisms that have definitively been ruled out as causal and highlighting those that are still plausible and thus merit further study.

The organization of this document mirrors the process by which a pathogen infects a host and causes disease in that host. The various elements in this process are each critical in fully understanding the pathogenesis of a particular disease. For most diseases, some of the elements are understood while others remain unclear. The elements of particular interest in understanding the pathogenesis of infectious disease are:

- Infectious agent
- Transmission vector
- Means of entry/site of entry
- Target tissue
- Adherence to/entry into cell
- Growth/multiplication
- Spread to other locations in body
- Injury mechanism
- Immune response
- Immune response avoidance and interference
- Phenotype (course of disease/symptoms)

Infectious agent

When investigating an infectious disease, we want to know what type of organism is involved, because we can then better understand the nature of the disease and what therapies might be most useful in combating it. The major classes of pathogens are bacteria, viruses, protozoa, algae, fungi, helminths, ectoparasites, and prions. Subclassifications within these classes are frequently based on microscopically visible

features. Many classification systems are now being analyzed at the genome level and adjustments are being made based on those findings.

It is important to note that most of the microorganisms currently present in the environment have not yet been cultured (estimates of uncultured microbes range as high as >99% of all microbial species). It is entirely likely that many of these species may be pathogenic to humans and the causes of human diseases. New techniques for sequencing genomic material from unidentified and uncultured pathogens may make it possible to discover new microorganisms and tie their presence to particular diseases.

Following are brief descriptions of the types of infectious agents known to be capable of causing disease in humans. Very few general conclusions can be drawn about the characteristics of diseases caused by a given type of infectious agent – the various organisms in each category usually represent a wide range of approaches to pathogenesis. Subsequent sections in this document provide more information about the virulence factors of specific organisms.

Bacteria
Bacteria are small unicellular organisms that are prokaryotic (lacking a nucleus), unlike other unicellular pathogens which all possess nuclei. Two kingdoms of bacteria exist: eubacteria and archaebacteria. These kingdoms differ in cell wall compositions (eubacteria cell walls contain peptidoglycan, archaebacteria cell walls do not) and environment (archaebacteria tend to live in extreme environments). The bacteria discussed in this document are all eubacteria; archaebacteria have not yet been found to cause human disease.

Bacteria are often grouped by features relating to their morphology or their life cycles. Some common classifications include:
- "Typical" bacteria (e.g., *Streptococcus* and *Salmonella*) – Cocci (spherical bacteria), bacilli (rod-shaped bacteria), and spirochetes (worm-like spiral-shaped bacteria) that are either Gram-positive or -negative (i.e., their cell walls contain either thick or thin peptidoglycan layers, respectively)
- Higher bacteria (e.g., *Nocardia* spp. and mycobacteria) – organisms possessing properties often associated with fungi such as the ability to form branching filaments
- Rickettsiae and ehrlichiae – Gram-negative obligate intracellular bacteria associated with animal reservoirs and/or arthropod vectors; endotoxin may be primarily responsible for disease
  - Rickettsiae (spotted fever group and typhus group) – have mammal reservoirs and arthropod vectors
  - Ehrlichiae – typically have a tick vector and a tropism for macrophages or granulocytes in which they grow inside cytoplasmic vacuoles
  - Coxiella (Q fever) – reservoirs are cattle, sheep and goats, transmission is via the air
- Chlamydia (e.g., *Chlamydia trachomatis*) – obligate intracellular Gram-negative parasites with a unique life cycle involving two stages, both of which must be passed through in order to reproduce (one form, called the elementary body, is adapted for extracellular survival and infection of cells; upon cell entry, chlamydia

convert to the reticulate body form which is adapted for survival and reproduction inside host cells)
- Mycoplasmas (e.g., *Mycoplasma pneumoniae*) – smallest known free-living forms, prokaryotes that lack a cell wall, cause infection primarily as extracellular parasites, and attach to the surface of ciliated and nonciliated epithelial cells.

Bacteria may also be classified by the type of toxins produced (Gram-positive bacteria typically produce exotoxins and the cell membranes of Gram-negative bacteria contain endotoxins), spore formation capability (Gram-positive bacteria only), oxygen tolerance (bacteria can be aerobes, anaerobes, facultative aerobes, or microaerobes), or ability to ferment lactose.

Viruses
Viruses are infectious agents consisting of RNA or DNA surrounded by a protein coat (capsid). They do not possess the ability to reproduce by themselves and thus must inhabit living cells and use their hosts' genetic reproduction and protein manufacturing processes in order to multiply. Some of the classification systems used to categorize viruses include:
- Type and structure of nucleic acid
  - RNA or DNA (RNA is referred to as sense, also known as positive sense or plus sense, if it can serve as mRNA; otherwise it is anti-sense, or minus sense or negative sense, if a complementary mRNA strand can be transcribed using it as a template)
  - Single- or double-stranded
  - Circular or linear
  - Single molecule or multiple discrete segments
- Geometric symmetry of capsids
  - Helical
  - Icosahedral
- Presence or lack of a lipid envelope from the host cell
  - Enveloped: sensitive to desiccation in the environment and often transmitted by respiratory, parenteral, and sexual routes
  - Non-enveloped: stable to harsh environmental conditions and often transmitted by the fecal-oral route

In addition to infecting humans directly by invading human cells and reproducing there, viruses can also cause disease when they inhabit other organisms that in turn become pathogens. For example, viruses that infect bacteria (and thus are known as "bacteriophages") have been found to cause disease as is the case in cholera.

Protists
Protists are animal-, plant-, or fungus-like organisms such as protozoa, algae, plankton, and slime mold. Among these organisms, protozoa are the most capable of causing disease in humans.

Protozoa are single-celled motile eukaryotic organisms with a complex system of organelles enclosed by a cell membrane. Protozoa assume different forms at different points during their life cycle. Infectious protozoa may live as either extracellular and intracellular parasites. They are classified by their morphological features:

- Flagellates/mastigophora (e.g., *Leishmania*) – use flagella and usually multiply by longitudinal binary fission
- Amoebae (e.g., *Entamoeba histolytica*) – pseudopod-forming, multiply by binary fission
- Sporozoans/apicomplexa (e.g., malaria parasites and coccidia) – move by bending, creeping or gliding; reproduce by multiple fission; often invade cells
- Ciliates (e.g., *Balantidium*) – use cilia to move, have apical complex and reproduce by transverse binary fission
- Other/unclassified

Protozoal diseases are often chronic, sometimes taking years to resolve.

Algae, another group of protists, occasionally directly infest humans (for instance, *Prototheca* causes protothecosis). However, more often their role in human disease is due to their production of harmful toxins which are taken up by fish and shellfish and cause food poisoning when ingested by humans.

Fungi
Fungi are eukaryotic, non-motile organisms that can be either unicellular or multicellular. They have rigid cell walls composed of chitin, mannans and occasionally cellulose. Their usual role in the environment is to break down dead organic material; however, some species are also capable of parasitizing living creatures. Since healthy people are generally able to resist infection by fungi, most fungal infections are found in immunocompromised hosts.

The two major classifications of fungi are:
- Yeasts (round or oval; reproduce by budding)
- Molds (made up of tubular structures called hyphae; grow by branching and longitudinal extension)

Some fungi do not fall neatly into either category – for instance, some fungi have features of both yeasts and molds. These are called dimorphic fungi and often only one of the two forms is pathogenic in humans. Factors such as temperature, CO_2 concentration, and pH determine which of the two forms a dimorphic fungus will assume. Therefore a fungus may be found in one form in nature but change to another form when invading host tissues; this ability to transform its morphology is thought to be a critical virulence factor.

Fungal infections are generally cutaneous, subcutaneous, or systemic in nature. Systemic infections result either from inhalation of fungal spores or particles or the proliferation of commensal fungi in immunocompromised hosts.

Animals
Animals that are small enough to parasitize humans fall into two categories, helminths and ectoparasites:
- Helminths (are multicellular, have complex reproductive systems, have egg, larval and adult stages, initiate infection by ingestion of eggs or penetration of intact skin by larvae)
  - Nematodes (roundworms, e.g., *Ascaris lumbricoides*)
  - Trematodes (flukes, e.g., *Schistosoma mansoni*)

- Cestodes (tapeworms, e.g., *Echinococcus granulosus*)
- Ectoparasites (e.g., scabies mites) – parasites that live on the outer surface of their hosts; these are generally arthropods (insects, arachnids, millipedes, centipedes, and crustaceans)

Because of their size and mobility, helminths and ectoparasites are capable of causing direct damage to tissues in the host.

Prions

Prions are protein particles that are believed to cause several transmissible species-specific neurodegenerative diseases such as Creutzfeld-Jakob Disease, although the exact etiology of prion diseases is unknown. Diseases caused by prions typically affect the central nervous system, have long incubation periods, and are generally fatal. They also are linked to an intracellular accumulation of a protease-resistant protein called PrP (prion protein), which is a component of prions and is hypothesized to be the responsible agent in these diseases.

Transmission vector

Knowing how an infectious agent reaches a new host is useful in understanding how to prevent transmission. Transmission vectors are often closely linked to how and where a disease agent first encounters its host – for instance, a pathogen in an airborne vector is likely to first encounter the respiratory system, while one using a liquid vector is more likely to be ingested. Some transmission vectors also are associated with specific epidemiological features of disease. For example, animal vectors may be localized to particular regions or climates while transmission by various types of social contact may be more highly associated with certain age groups.

Common transmission vectors implicated in human disease include:
- Air particles, aerosols (sneezing, skin shedding)
- Food/drink (fecal contamination, food spoilage, human milk)
- Liquids (contact lens solution, water used for swimming or washing)
- Body fluids
  - Blood (via needle sticks, insect bites, conflicts, accidents, maternal transmission to fetus)
  - Saliva/mucus (via bites, spitting or social contact)
  - Other (semen, placental fluid, etc.)
- Direct contact between skin and skin or other surfaces

Infectious agents are often closely associated with a small number of vector types appropriate to their needs and abilities. For instance, as mentioned above, enveloped viruses are not stable in the environment for long and thus must be transmitted by vectors that can bring viruses from an infected person to a new host in a short period of time. Although vectors are often just passive carriers of the pathogen from one host to the next, occasionally they play a more dynamic role in the pathogen's life cycle. For example, *Trypanosoma cruzi*, the protozoa that causes Chagas disease, goes through

two transformations in its bug vector before infesting a new human host (whereupon it undergoes two more transformations).

Site of entry/means of entry

A factor in pathogenesis that is closely related to the transmission vector is the site at which the infectious agent gains entry to a new host. Knowing the typical entry sites for a given agent is again useful in preventing further transmission as well as in understanding how and where the disease begins to get established in the host.

Common sites of entry and the means by which the organism gains entry into the host include:

Site of entry	Means of entry
Skin	Small bites (insects), large bites (animals) Needle sticks Cuts/wounds/burns Surface contact/direct invasion (e.g., hookworm larvae can burrow directly through the soles of a person's feet)
Respiratory system	Inhalation
Oropharynx	Ingestion, inhalation
Gastrointestinal tract	Ingestion
Urinogenital tract	Entry through urethra enabled by ineffective flushing of urine Sexual contact
Blood/internal organs	Organ, blood or tissue transplant/transfusions
Conjunctiva (membranes covering the outer eye and inner eyelid surfaces)	Introduction via fingers, contaminated instruments, swimming, etc.

Normally we think of entry taking place in a new host who is independent from the transmitting hosts. However, a fetus can also be infected by his/her mother through a number of the sites listed above (gastrointestinal tract, conjunctiva, skin), through transmission events taking place either *in utero* or during the birth process.

Some pathogens tend to be associated with one particular site of entry; others are known to enter the body and initiate infectious activities at multiple sites. For example, rabies virus almost always enters through the skin (as the result of a bite), whereas *Chlamydia trachomatis* can enter the body through both the conjunctiva and the urinogenital tract, leading to ocular and genital infections respectively.

Target tissue

Pathogens tend to be specialized to live in particular types of environments. Each pathogen has a set of survival mechanisms and requirements for growth and reproduction, and each is successful only when it reaches a tissue where the defense mechanisms can be overcome, at least temporarily, where its environmental requirements (e.g., temperature, pH, and oxygen) are met, where there are cells with a sufficient number of the appropriate receptors, and where the appropriate nutrients and other resources can be found. Some pathogens are fairly flexible and can live in a range of tissues; others are limited to a small number of inhabitable areas.

Almost any tissue in the body (even toenails) can be a site of infection. Listed below are a number of sites that can serve as targets for pathogens, the barriers to infection that they present, and a few examples of the pathogens that nevertheless find these sites to be hospitable.

Skin
Intact skin is normally inhospitable to pathogenic microorganisms due to dry, slightly acidic conditions, the presence of commensal organisms, continual shedding of the top layer, etc. Therefore, breaks in the skin caused by cuts, abrasions and other wounds are often required for infection to take place. Infection may be localized to the dermis, epidermis or hair follicles. Examples of organisms that infect the skin include:
- *Staphylococcus aureus*
- Scabies
- Papilloma virus (warts)
- Tinea pedis (athlete's foot fungus)

Respiratory system
Organisms who can successfully establish themselves in the respiratory system must be capable of binding tightly to epithelial cells to avoid getting caught up in mucus, being removed from the lungs by the mucociliary elevator, and swallowed. They also must avoid being phagocytosed by alveolar macrophages unless they are capable of surviving phagocytosis.
- *Bordetella pertussis* (whooping cough)
- Influenza virus
- *Aspergillus* species (aspergillosis)

Gastrointestinal tract
A variety of pathogens can inhabit the stomach, intestines, and other GI organs. One barrier to colonization is the constant flow of material through the system; another is the population of commensal, non-disease causing bacteria competing for resources. A mucus layer coating the epithelium makes attachment difficult for many pathogens; therefore a common pathway for invading the intestine is via the M cells, whose function it is to take up antigens and pathogens for presentation to other immune system cells.
- *Escherischia coli*
- *Shigella* species (shigellosis)

- *Entamoeba histolytica* (amebiasis)
- *Helicobacter pylori* (gastritis, ulcers)

Oropharynx

The mouth and throat are continually exposed to airborne pathogens and thus are common sites of infection. However, pathogenic organisms infecting this region must both compete with normal oral flora and avoid the defense mechanisms provided by saliva. Therefore they gain an advantage in situations where the normal flora levels or salivary output are depressed.

- *Streptococcus mutans* (dental caries)
- *Streptococcus pyogenes* (strep throat)
- *Candida albicans* (thrush)

Circulatory system

Many pathogens use the bloodstream to spread from site to site; however, some make the circulatory system their primary home. Some resident pathogens invade blood cells such as monocytes or red blood cells. Others colonize circulatory tissue, especially damaged or abnormal tissue where the blood flow that would normally prevent attachment is disrupted.

- *Rickettsia rickettsii* (Rocky Mountain spotted fever)
- *S. aureus*
- *E. coli*
- *Plasmodium* species (malaria)

Urinogenital tract

The primary defense against microorganisms in the urethra is the regular outward flow of urine; infection therefore requires either a blockage of urine or the ability to bind tightly to epithelial cells to avoid being swept away. Because the urethra is much shorter in women than in men, women are much more likely than men to contract urinary tract infections. In contrast to the urinary tract which is mostly sterile, the female genital tract is heavily populated with commensal bacteria. These bacteria actually help to prevent infection by generating an acidic environment that pathogens attempting to colonize the reproductive tract must be capable of withstanding.

- Uropathogenic *E. coli*
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*

Conjunctiva

The conjunctiva is normally very resistant to infection due to the continual mechanical sweeping away of particles by the eyelid as well as the various immune system components found in tears. However, damage caused by rubbing, foreign objects, swimming or trauma may create protected sites where a bacterial or viral infection can become established.

- *S. aureus*
- *Chlamydia trachomatis*
- Adenoviruses

Nervous system

The nervous system is normally protected from direct invasion due to its location inside the body, and the brain is additionally protected by the function of the blood-brain barrier. Nevertheless, pathogens can reach the nervous system through bloodstream transmission from other sites. Wounds (surgical or traumatic) may also expose nerves and brain tissue to pathogens in the environment. Infectious agents may spread along the nervous system – for instance, some central nervous system invasions such as varicella-zoster are facilitated by spread along the peripheral nervous system.

- *Angiostrongylus cantonensis* (nematode that invades the brain in larvae form)
- *Neisseria meningitidis* (meningitis)
- *Trypanosoma brucei* species (sleeping sickness)
- Herpesviruses

Immune system

Invasion of immune system tissue and cells is a useful strategy on two counts: first, it provides the pathogen with a protected place to live, and second, the invasion itself may weaken the immune response so that the organism faces fewer threats to its survival. Specific targets include T cells, B cells and macrophages, among others. Pathogens that are adapted to survive inside phagocytic cells can take advantage of the fact that the function of these defenders is to engulf foreign bodies.

- HIV
- *Ehrlichia chaffeensis* (human monocytotropic erlichiosis)
- Epstein-Barr virus (infectious mononucleosis)

Adhesion to host cells

Attachment to cells or extracellular matrices is an important part of the pathogenesis of infectious diseases. Pathogens are much more vulnerable to immune defenses and flushing mechanisms when they are living freely in body fluids. Adhesion is mediated by ligands (adhesins), which are molecules or projections on the surface of the pathogen that bind to receptors on the surface of host cells. Pathogens tend to attach only to certain types of cells, leading to the conclusion that adhesins and receptors are highly specific. (This may account for the tissue tropism seen in most diseases.) A few pathogens are capable of attaching to a wide variety of cells – this is accomplished through the expression of different adhesins or complexes at different times to target various tissues.

Features that enable attachment to host cells include pili (fimbriae), non-pilus adhesins (afimbrial adhesins), and other structures.

Pili

Pili are thin hair-like protein projections found on some bacteria. They have an adhesive tip whose shape fits specifically with a host cell receptor. Two species of bacteria that use pili are:

- *Neisseria gonorrhoeae,* which can synthesize a variety of pili with different adhesive tips to target different cells

- Uropathogenic strains of *E. coli,* whose pili allow the bacteria to attach strongly to the urinary tract epithelium so that they avoid getting washed away by urine

Non-pilus adhesins

Non-pilus adhesins are molecules, usually proteins but sometimes polysaccharides, on the surface of a pathogen that bind specifically to host cell receptors or other molecules to allow close contact. Two examples of bacteria that use non-pilus adhesins are:
- *Streptococcus pyogenes,* whose adhesin proteins F1 and M1 bind to soluble and extracellular matrix-associated fibronectin, which then facilitates adherence to host cells
- *N. gonorrhoeae,* whose Opa outer membrane proteins enable it to make a more intimate contact with the host cell after it first adheres with its pili.

Viral adhesins that mediate attachment come in various forms:
- Single capsid components that extend from the virion surface (adenovirus, reovirus, and rotavirus)
- Surface glycoproteins of enveloped viruses (influenza virus and HIV)
- Capsid proteins that form binding pockets to engage cellular receptors (poliovirus and rhinovirus)
- Capsid proteins that form loop-like structures for binding receptors (foot and mouth disease virus)

Some protozoa and fungi also use adhesins to bind to cells:
- *E. histolytica* trophozoites adhere to colonic tissue through a galactose-inhibitable surface lectin (a surface protein with a heavy and a light subunit)
- *Plasmodium* sporozoites use region II of circumsporozoite protein on their surface as a ligand to attach to receptors on hepatocytes; other ligands are probably used to attach to red blood cells
- *Candida* makes use of at least four different recognition systems involving adhesins composed of mannoprotein or mannan to bind to different cells

Other adhesive features

Biofilms: Some bacteria form a sticky external substance which helps them to adhere to cells as well as inert materials (such as medical implants) in layers called biofilms. Initial attachment of bacteria to a surface may be either specific or non-specific; subsequent production of exopolysaccharides secures the attachment of the bacteria to the surface and to each other. Dental plaque formed by *Streptococcus mutans* is a good example of a biofilm composed of bacteria and secreted capsular material.

Host cell structures: Instead of simply relying on its own components to bring about adhesion, *E. coli* goes one step further by inducing changes in the host cell that allow attachment. It causes the host cell to form a specialized actin structure (called a pedestal or pseudopod) to which the bacterium can then bind.

Adhesive structures: The protozoa *Giardia lamblia* has a concave adhesive disk that allows it to attach to intestinal epithelial cells, where it remains (providing an example of non-invasive attachment). Flukes have oral and ventral suckers that are used to attach to tissues.

Invasion of host cells

In addition to attaching to host cells, many pathogens also need to invade these cells. This is an absolute requirement for viruses, who need to enter a cell in order to replicate. Other infectious agents find the interior of host cells to be not only a safe haven for avoiding antibodies and other defense mechanisms but also a supply of necessary resources for growth and reproduction. Invasion is necessary for virulence in some bacteria but not in others. Invasion is of varying significance even within a species – for instance, some strains of *E. coli* are invasive while others are not. Those pathogens who are capable of living and reproducing outside the cell must possess means of evading extracellular immune defenses such as antibodies.

There are a wide variety of mechanisms for invading cells: a pathogen's surface can integrate itself directly with the cell's surface; the cell can take in pathogens along with a substance it needs; the cell's function may be specifically to take in pathogens (as is the case with macrophages); or a pathogen can force its way in.

Some of the invasion strategies used by various types of pathogens are as follows:

Viruses
Viral invasion mechanisms are based on whether the virus is encapsulated or not. Encapsulated viruses (such as hepatitis B) enter cells by fusing their viral envelope with the cell membrane or through inducing the formation of clathrin-coated pits (specialized structures on the cell surface that function in endocytosis) in the cell membrane. Non-encapsulated viruses (such as adenoviruses) enter either through endocytosis (uptake of receptor-bound material through the formation of a vesicle) or direct penetration.

Bacteria
Bacteria use a diverse range of mechanisms to invade cells. These mechanisms include:
- Induction of phagocytosis by microorganisms that inhabit phagocytes. Pathogens that use this strategy can take advantage of easy access into the host cell but must have some way of surviving the lethal processes inside the cell. For example, species of *Mycobacterium, Salmonella* and *Legionella* are all capable of surviving and multiplying within macrophages, for instance by inhibiting the phagosome in which they reside from maturing to the phagolysomal stage.
- Bacterial-mediated endocytosis, whereby bacteria induce endocytosis by non-phagocytic cells. Host cells who are not professional phagocytes may nevertheless be induced to take in bacteria by certain stimuli. *Salmonella* secretes proteins that induce structural changes (e.g., membrane ruffles) in the epithelial cell membrane and cytoskeleton, creating a hollow for the organism so that it can be taken in by macropinocytosis. *Shigella* invades cells using a similar mechanism.

- <u>Invasion of neighboring cells.</u> Some bacteria (e.g., *Shigella* species) can invade only the basolateral (side and bottom) surfaces of epithelial cells and not the brush borders, so they either invade neighboring M cells or induce transepithelial migration of neutrophils which may enable bacteria to penetrate the top barrier and get to the desired surface.
- <u>Use of motility factors.</u> Motility structures may be helpful in completing the invasion of a host cell. For instance, corkscrew action in conjunction with an invasin may help the spirochetes *Borrelia burgdorferi* and *Treponema pallidum* to invade cells.

Protozoa

Many protozoa are extracellular but there are some species that inhabit cells. Some, like *Leishmania,* cannot invade cells directly but instead depend on being phagocytized. Others are capable of direct invasion. For instance, malaria merozoites have a sophisticated multi-step process for entering cells. They (1) orient their apical end toward the host cell membrane, (2) discharge organelles called micronemes to form a tight junction with the membrane, (3) clear away the erythrocyte's cytoskeleton and form a vacuole, (4) drag themselves into the cell through the tight junction via myosin motors, and (5) then close up the vacuole and cell membrane behind themselves.

Fungi

Fungi generally live on cells, not in cells, unless they are engulfed by phagocytes. *Cryptococcus neoformans* is a facultative intracellular parasite that inhabits macrophages; *Paracoccidioides brasiliensis* appears to be as well.

Helminths

Helminths are usually too large to inhabit cells. An exception is *Trichinella spiralis* whose larvae live in muscle cells.

Growth and multiplication

The ultimate goal of a pathogen is to successfully grow and multiply, creating offspring that can then leave the body to infect other hosts. Most human pathogens (with the exception of most helminths) are able to replicate in their host; some pathogens (such as viruses) are even obligated to be inside a host to replicate. As discussed above, some microorganisms need to inhabit a cell to reproduce while others can do so extracellularly. In order to grow and multiply, pathogens also must have access to nutrients and other essential resources.

Each type of organism has unique requirements for reproduction and mechanisms for doing so.

Viruses

Viruses replicate themselves using the protein manufacturing machinery of the cell. Replication involves not only replicating the viral genome but also transcribing mRNA segments that are used to produce the protein components that assemble to form the

capsid. Replication requires that the genome first be partially or completely uncoated, or extricated from the capsid, so that expression can take place. It also requires that the cell infected by the virus be permissive, or capable of producing infective progeny (all cells are not permissive for all viruses – for instance, through lack of necessary transcription or replication factors for that virus).

The specific steps involved in viral replication depend largely on the virus's nucleic acid type. Variables in the process include whether essential processing components must be included as part of the virion and whether replication occurs in the nucleus or the cytoplasm.

- Positive-sense single-stranded RNA that can be used directly as mRNA (e.g., picornaviruses, togaviruses)
    - Cellular ribosomes translate the RNA after the virus's entry into the cytoplasm
    - One of the translation products is RNA polymerase which replicates the viral RNA (after the creation of a (-)sense RNA intermediate to act as a replication template)
- Negative-sense RNA (e.g., orthomyxoviruses, rhabdoviruses)
    - Virions must contain a pre-formed RNA polymerase to transcribe (+)sense mRNAs
    - Replication also requires creation of a (+)sense copy to serve as a template
- Double-stranded RNA (reoviruses)
    - Virions include RNA polymerase that catalyzes the synthesis of (+)sense RNA molecules using the (-)sense strand as a template
    - Transcribed (+)sense RNA molecules are either translated into viral proteins or assembled into a complete (+)sense strand onto which a (-)sense complementary strand is assembled to form a complete progeny genome
- (+)sense RNA with a DNA intermediate (retroviruses)
    - RNA does not serve as mRNA but instead as the template for the synthesis of a double-stranded DNA copy called the provirus
    - Synthesis of the provirus is mediated by a virus-encoded reverse transcriptase
    - Provirus is translocated to the nucleus and is integrated into host chromosomal DNA
    - Virus is replicated through transcription of the integrated DNA
- Double-stranded DNA
    - The genomes of some DNA viruses (papovavirus, adenovirus and herpesvirus) are transcribed in the nucleus using the cell's enzymes
    - Other DNA viruses (e.g., poxviruses) are replicated in the cytoplasm (presumably they provide their own transcription factors)
- Single-stranded (+) and (-)-sense DNA (parvoviruses)
    - Replication occurs in the nucleus
    - Creation of a (-)-sense strand is required to provide a template for mRNA synthesis

Bacteria
To grow and reproduce inside the human body, bacterial pathogens require a number of resources: nutrients, a source of energy, water, and an amount of oxygen suited to their needs. Some bacterial pathogens reproduce intracellularly – sometimes within a vacuole within the cell, sometimes not. Other bacteria are capable of reproducing extracellularly. The normal mechanism for bacterial reproduction is binary fission. Reproduction is asexual; however, some bacteria possess conjugative plasmids through which they are able to connect to other bacteria, initiating a one-way transfer of genetic material (plasmids or chromosomal genes) to their counterpart.

Protozoa
Like bacteria, protozoa also typically reproduce by binary fission, although some may also multiply through budding or multiple fission. Protozoa often have multi-stage life cycles. For instance, intestinal protozoa such as *E. histolytica* and *Giardia* are ingested by humans in cyst form. Once inside the digestive system of the host, the cysts transform into trophozoites (the form which actively feeds and multiplies). These then reproduce asexually to create new trophozoites. Some trophozoites develop into cysts and leave the host, thus completing the cycle. Protozoa belonging to the phylum Apicomplexa often have even more complex life cycles and may reproduce sexually as well as asexually.

Protozoa have metabolic requirements similar to those of their hosts (preformed organic substances). Their parasitic feeding activities and growth often lead to cell and tissue damage in the host. They may also cause changes in cell function and morphology. For instance, malaria parasites have been found to induce new permeation pathways in infected red blood cells to meet their metabolic needs.

Fungi
Reproduction in fungi takes place through both sexual and asexual mechanisms. Yeast reproduce and thus expand in the host by asexual budding, or mitotic binary fission. Molds begin life as spores, which after germination become forms called hyphae that grow through branching. Some molds are capable of sexual reproduction to form sexual spores; others can only reproduce asexually, forming asexual spores called conidia.

Fungi obtain nutrients by absorbing them through their cell wall. Small compounds can be absorbed directly; others are first broken down by digestive enzymes that the fungus releases through the cell wall.

Helminths
Most helminths that infect humans do not increase their numbers in the host; instead, the offspring they produce exit the infected person, often through feces. Exceptions to this general rule include *Strongyloides stercoralis* and *Echinococcus*. Roundworms and blood flukes are bisexual; other flukes and tapeworms are hermaphroditic.

All types of helminths except for the tapeworms possess an alimentary canal and a mouth through which they take in food. Tapeworms absorb nutrients across their microvilli-covered surface.

Prions

It is not yet known how prions reproduce or replicate in human hosts. One hypothesis is that they induce transformational changes in normal proteins so that they too become prions. Additional research is required before we can know the exact mechanism for prion multiplication.

Spread to other locations in body

While some pathogens remain localized to their original place of infection at the site of entry, it is also common for pathogens to move into other locations in the body that prove to provide a more suitable environment. Some pathogens are capable of moving on their own from site to site; others take advantage of a host component that is already moving throughout the body (e.g., blood or lymphatic fluid). In some cases, lysis or apoptosis of host cells is required to release progeny so that they can spread into new cells or tissues.

Common means of traveling into neighboring or distant tissues include:

Invading neighboring tissues directly

Listeria monocytogenes moves directly into neighboring cells, propelled by actin structures synthesized within the host cell that push the cell membrane into a pseudopod-like protrusion that is then engulfed by the adjoining cell. Cell-to-cell spread is also possibly facilitated by lysing the host cell membrane with its neighbor's membrane. The direct invasion capabilities of this bacterium may, for example, account for its ability to move through the microvascular blood-brain barrier into the central nervous system. Other pathogens are able to move throughout the body on a larger scale – for example, many helminths migrate broadly from one site to another, often causing damage as they go.

Traveling through the lymphatic system

Some pathogens are capable of inhabiting lymphoid cells without setting off a rapid immune response, and in certain cases are even able to multiply in lymph nodes. For these, the movement of lymphatic fluid is an ideal way to reach other parts of the body. This is an especially important mechanism for viruses such as measles virus and HIV, many of which can invade lymphoid cells without causing immediate cell damage.

Traveling through the blood

Infectious agents can travel as bloodborne pathogens either in the plasma or in association with leukocytes, erythocytes, or platelets. As bloodborne agents, they can be taken up by phagocytic cells in various organs, localize in tissues where blood flow is slow, or cross a blood-tissue boundary (e.g., the blood-brain barrier). An infection can move through the bloodstream multiple times leading to multiple infections. A primary viraemia or bacteraemia can lead to localization in one tissue, then a secondary viraemia/bacteraemia can lead to localization in a different tissue, and so on. For instance, the amoeba *E. histolytica* can spread from the intestines via the portal vein to

the liver, and from there also to the lungs or skin. This process of repeated blood infections can result in long incubation periods involving multiple sites in the body. The presence of pathogens in the blood can be symptomatic or asymptomatic, depending on factors such as the number of agents in the blood and whether or not they release toxins into the bloodstream.

Spreading through the peripheral and central nervous system
Infectious agents and their toxins can spread through the central nervous system by entering the cerebrospinal fluid, by transversing the blood-brain barrier, and by traveling up and down peripheral nerves. An example of a pathogen disseminating through the nervous system is the rabies virus. This virus comes into contact with peripheral nerves through a break in the skin, travels up the nerves into the brain, and then spreads back along the nerves to the salivary glands. Herpes simplex virus is another virus that travels along the peripheral nerves.

Spreading via the pleural and peritoneal cavities
Injuries or infections of organs can sometimes cause breaches in the integrity of organ surfaces, leading to infection of internal cavities that can result in the rapid spread of microorganisms to other internal sites. These cavities are lined with macrophages and polymorphs whose role it is to inhibit the establishment of new infections, so presumably pathogens that are capable of infecting these cavities must possess virulence factors that enable them to evade these defenses. *E. coli* that normally resides in the gastrointestinal tract is an example of a pathogen that is able to colonize the peritoneal cavity.

Cause of injury

Essential to understanding the pathogenesis of a disease is knowing what causes the damage to the body's cells and tissues and what produces the symptoms experienced throughout the course of the illness. Knowing the basis of the injury may lead to therapies for preventing or alleviating the damage.

There are many known mechanisms by which diseases cause damage. Some result directly from the activities of the infectious agent, other are consequences of the body's response to the agent.

Direct damage to host cells
Pathogens often directly damage the cells that they inhabit or come into contact with; sometimes they even damage remote cells through the release and spread of toxins. In some cases the reason for this damage is clearly related to the pathogen's needs – for instance, a pathogen inhabiting a cell may need to lyse its host in order to escape. In other cases it is less clear what, if any, advantage is gained by a pathogen's toxic activity, given that it would seem to be in an organism's best interest to cause as little damage to its host as possible. Cellular damage, if widespread enough, may be a problem in and of itself; direct damage may also instigate inflammatory or immune

processes that wind up causing further injury to the host. Means by which infectious agents directly harm host cells include:

- Induction of apoptosis (programmed cell death) by viruses. Certain viruses, such as HIV, adenoviruses, herpesviruses, influenza virus and picornaviruses have been shown to induce apoptosis in host cells. Often the induction of apoptosis is a host defense strategy to destroy infected cells, thus halting viral production. In fact, many viruses possess factors that inhibit or delay apoptosis to allow continued viral replication. Sometimes apoptosis appears to be a viral strategy. Possible reasons for this activity include escape from the cell in late stages of the infection and destruction of immune cells that would otherwise destroy the virus. For instance, the viral protein PB1-F2 associated with the influenza A virus is thought to localize to the mitochondria in immune cells and induce apoptosis, thus possibly helping to defuse the immune response.
- Production of cell-damaging toxins. Substances produced or released by pathogens often have the effect of damaging the cell in which the bacteria reside (*E. coli*) or to which they attach (mycoplasma, *Entamoeba histolytica*). Some toxins, such as amoebapore produced by *E. histolytica*, act on cell membranes to lyse the host cells. Others damage internal membranes and structures. For example, listeriolysin O lyses phagosomes, allowing *Listeria monocytogenes* to escape, and *E. coli* cytotoxic necrotizing factor type 1 causes necrosis by causing actin stress fiber formation and inducing membrane ruffles in the host cell. Some toxins are capable of causing widespread harm when released into the bloodstream. For instance, although *Corynebacterium diphtheriae* tends to only colonize the nasopharyngeal cavity and the skin, the diphtheria toxin (which is actually encoded by a phage) can spread via the bloodstream throughout the body, affecting in particular the nerves and the heart.
- Competition through using up or diverting cellular resources. Pathogens that inhabit cells compete with the cell's own functions for resources such as energy and synthetic machinery, sometimes to the detriment of the cell. Viruses, for example, must divert the cell's replicative and synthetic machinery to their own uses if they are to multiply; this diversion can deprive the cell of the ability to synthesize needed macromolecules. One way in which this is done is through controlling the synthesis of certain host proteins, for instance through binding cellular transcription factors or cleaving molecules involved in translation. For example, enteroviruses such as poliovirus and coxsackie virus encode a protease that cleaves a host protein involved in the translation of cellular proteins. The loss of protein production eventually leads to the cell's death.
- Mechanical lysis. It is thought that *Rickettsia prowazekii* destroys the endothelial cell in which it multiples simply by bursting it open due to the accumulation of large numbers of progeny bacteria.
- Ingestion of host cells and tissues. Certain helminths are capable of ingesting host tissues to the extent that symptoms are induced. For example, hookworms attach to the mucosal lining of the intestine and proceed to feed on mucosal tissue and blood. Sufficient blood loss through ingestion and through leakage from the attachment sites can lead to anemia and other manifestations.

Damage to extracellular structures or processes
In addition to damaging cells, pathogens can also damage structures and systems outside the cell. One way is by liberating toxins into extracellular fluids that damage the

surrounding area. For instance, the enzymes elastase and alkaline protease released by *Pseudomonas aeruginosa* cleave structural proteins such as collagen and fibrin, and *E. histolytica*'s proteinases appear to dissolve the extracellular matrix anchoring cells and tissue structure. Toxins can also interfere with extracellular processes such as extracellular signaling (botulinum toxin and tetanus toxin both cleave proteins necessary for neurotransmission).

Physical interference with organ or vessel function
Another means of direct damage to physiological processes is the blockage of organs or vessels by pathogens or pathogen-infected cells. For instance, helminths, due to their large size, can block internal organs or vessels, thereby increasing internal pressure and causing blood loss. They can also damage tissue during their migration through body or induce granulomatous responses that result in blockage (this is how schistosome eggs cause liver pathology, for instance). Malaria parasites bring about blockage intracellularly. They modify their host red blood cells to produce surface knobs that cause the cells to adhere to capillaries and small veins, preventing their elimination by the spleen and leading to microvascular disease.

Indirect damage via inflammation
Inflammation is a process characterized by dilation of capillaries, migration of leukocytes, heat, redness, pain and swelling; its purpose is to help clear infectious agents and infected or damaged tissues. In some cases the inflammatory response itself results in further injury which can be either transitory or chronic in nature. For example, the inflammatory response to a streptococcal infection may result in both tissue damage and chronic lesions. Macrophages may have difficulty digesting the bacteria and therefore may leak enzymes that destroy tissue, or die and become the basis of lesions. It is also thought that in severe infections caused by Gram-negative bacteria, release of endotoxins stimulates the release of cytokines in excessive amounts, leading to harmful effects such as respiratory distress and vascular collapse. Inflammation can also have indirect effects on the health of a host. For example, it has been hypothesized that inflammation plays a role in the maladsorption of nutrients seen in giardiasis.

Indirect damage via immunological factors
Just as a strong inflammatory response to an infection can result in injury, so can an excessive or inappropriate response by immune cells. Indirect effects caused by stimulation of immune forces include:
- Anaphylactic reactions (type 1 hypersensitivity). Allergic reactions which are mediated by IgE and mast cells are often triggered by allergens such as grass and pollen, but they can also be set off by the proteolytic enzymes secreted by helminths. Large-scale release of histamine from mast cells activated by antigen-antibody compounds can lead to anaphylactic shock characterized by rash, asthma, and even respiratory distress.
- Cytolytic or cytotoxic reactions (type 2 hypersensitivity). Antibody-dependent cellular cytotoxicity (ADCC) is an immune mechanism involving the destruction of

infected cells. It is thought that this process, when carried out to an extreme, may result in the liver necrosis seen in hepatitis B and yellow fever.

- Immune complex reactions (type 3 hypersensitivity). When antigen is present in large amounts or persists over long periods of time, the complexes it forms with antibodies can deposit in the tissues. These deposits may lead to damage, for instance through invoking an inflammatory response or through combining to form aggregates which alter the tissue structure. Diseases caused by persistent pathogens (such as malaria) may result in type 3 hypersensitivity.

- Cell-mediated reactions (type 4 hypersensitivity). Type 4 hypersensitivity is characterized by an overly vigorous T cell response, whereby activated CD4+ T cells release cytokines to stimulate inflammation and attract an abundant number of lymphocytes and macrophages. The body's reaction to poison ivy is a common example of type 4 hypersensitivity. Two possible effects of type 4 hypersensitivity in infectious diseases are granulomas induced by indigestible materials and tissue damage caused by ingestion of infected cells. Tuberculosis and leprosy may involve type 4 reactions.

- Reaction to superantigens. Superantigens are toxins that bind to MHC class II molecules in a way that leads to non-specific activation of T cells, thereby stimulating an enormous percentage (up to 20%) of the infected person's T cells. This overstimulation of T cells results in an excessive release of cytokines, ultimately affecting many biological systems, interfering with a coordinated immune response, and potentially leading to shock or death. *Staphylococcus aureus* and *Streptococcus pyogenes* both produce toxins that are known to be superantigens.

- Autoimmune activities. Some infections give rise to an autoimmune disease when the antibodies produced in response to the infectious agent also react with the body's own cells and tissues. One example of a disease that may be autoimmune in nature is rheumatic fever. This disease has been proposed to result from antibodies formed to attack streptococcal antigens that subsequently attack similar antigens in the patient's heart muscle or valves.

Induction of tumors by viruses

Viruses such as the leukemia viruses and human papillomaviruses cause damage by turning normal cells into tumor cells. Tumors may be induced by viral introduction of a transforming gene into the cell's DNA. Viruses may also encode proteins that block apoptosis or induce cell-cycle progression.

Immune response to infection

An infectious agent trying to establish itself in a host will normally trigger a response from the host's immune system. The nature of this response and its success in controlling and/or eliminating the infection will depend on the pathogen itself as well as the competence of the host's defenses.

Most illnesses elicit a barrage of different responses (antibodies, inflammation, cell-mediated responses, etc.) that work together in different ways over time to respond to

the infection. For example, the defense mechanisms triggered in whooping cough (caused by *Bordetella pertussis*) include increased numbers of B and T cells, inflammation, macrophage activity, and production of interferon. For any given disease, a particular defense system may play either a major or a minor role, and it is of great use to identify which defenses are necessary in clearing the infection. Some defenses play an important role in a wide range of illnesses, while others are particularly effective against a smaller, targeted set of pathogens.

An important distinction to make is that between innate and adaptive immunity. Some defenses (such as inflammation, complement, natural killer cells, and phagocytic cells) are present from birth and not specific to any organism; these general-purpose forces are activated at the first sign of infection and constitute a person's innate immune defenses. Other defenses (T cells, B cells, and antibodies) provide us with adaptive (or acquired) immunity – they target specific antigens and thus are highly effective at combating specific pathogens. An acquired immune response involving the proliferation of antigen-specific T and B cells may take several days or weeks to develop during an initial exposure to a pathogen, but is activated much more quickly on subsequent exposures, in some cases so effectively that the pathogen never has a chance to establish a second infection in the same host.

In this section we briefly describe the cells and molecules that together comprise the immune system, and then we show how they work in cooperation to achieve the body's goal of controlling infection.

Immune system cells
Immune system cells conduct a number of activities in response to an infectious agent, such as releasing molecules to stimulate additional activity, engulfing and digesting the pathogen, and manufacturing antibodies against the pathogen or its products. The nature and stage of the infection dictate which types of cellular activity will be most potent.
Types of cells involved in immune responses include:
- Myeloid cells (nonlymphocytes)
    - Neutrophils: Phagocytic cells that respond to chemotactic signals released by microorganisms and/or immune cells and migrate into infected tissue through inflammation-induced vascular permeability. Once in the affected area, they bind to and phagocytose (engulf and digest) the microorganism. Neutrophil deficiencies can lead to recurrent bacterial and fungal infections.
    - Macrophages/monocytes: Phagocytic cells that live in tissues or blood. Besides engulfing microorganisms, they also process foreign peptides and present antigens to other immune cells (e.g., T cells) to activate them.
    - Eosinophils: A cell that attacks large parasites (e.g., helminths) by releasing substances from granules that damage the parasite membrane. Eosinophils are also involved in allergic reactions.
    - Basophils and mast cells: These cells release histamine-rich granules and are also often involved in allergic reactions.
- Lymphoid cells (lymphocytes)

- CD4+ cells (Th, helper T cells): Once activated by antigen presented by other immune cells in conjunction with Class II MHC molecules, these cells release cytokines which stimulate and coordinate other immune activities. T cell response time varies for different pathogens (a few days up to several weeks). CD4+ cells are divided into different groups (Th1, Th2 and Th0) based on the types of cytokines they release. Deficiency in CD4+ cells can lead to a variety of opportunistic infections caused by microorganisms such as *Pneumocystis carinii*.
- CD8+ cells (Tk, killer T cells): CD8+ cells recognize tumor cells and virally infected cells (through the expression of Class I MHC molecule/viral protein combinations) and release chemicals that kill them. CD8+ cell deficiency is also associated with opportunistic infection.
- B cells: B cells include memory cells that process and present antigen to activate T cells, and plasma cells that manufacture antibodies (see below). They are activated by cytokines released by CD4+ T cells.
- Natural killer cells (NK cells): These cells lyse virally infected cells, tumor cells and microorganisms. They are stimulated by the presence of interferon released by virally infected cells and are often the first line of defense against viral infections.

Immune system molecules

Various types of molecules are synthesized and released by cells to counteract infections. These molecules interact with other molecules, including receptors on the surface of cells, to stimulate other defense forces or neutralize infectious agents directly.

- Antibodies. Antibodies are molecules manufactured by plasma B cells that bind specifically to antigens on pathogens or antigens released by pathogens. They play an important role in a number of diseases, such as systemic diseases and diseases caused by toxins (such as diphtheria). Different forms of antibody (IgM, IgA, IgG, and IgE) are produced at different times and places and are effective in different situations. For instance, IgM is produced during the first exposure to an antigen and IgG is usually formed in subsequent infections with the same antigen. Antibodies generated in response to the antigens produced by a pathogen may have very high affinity, thereby protecting strongly against infections, or they may have low affinity, providing little acquired protection.
- Complement. The complement system is a group of extracellular proteins that, when stimulated, work together to attack microorganisms directly or boost the activity of other immune functions, for instance by amplifying the inflammatory response to an invading pathogen (e.g., *Aspergillus*). The complement system can be stimulated either by antibody-antigen complexes (the classic pathway) or by microbial antigens alone (the alternative pathway).
- Cytokines. Cells signal each other by releasing molecules called cytokines; when these molecules encounter a target cell, they bind to high affinity receptors on the cell's surface, inducing that cell to initiate a specific activity. Cytokines may be released by immune cells (particularly CD4+ T cells and macrophages) or other cells involved in a response to infection. Some of the cytokines commonly seen in immune responses include:
  - Interferons (alpha, beta and gamma) are a family of glycoproteins produced by infected cells and immune cells in response to infection, particularly viral infection. Interferons bind to receptors on the

membranes of nearby cells in a manner which stimulates the production of enzymes inhibiting viral replication, thus protecting these cells from infection. Interferons also stimulate the activity of immune cells such as macrophages.

- o Interleukins are a increasingly growing group of regulatory proteins released by T cells and macrophages to modulate inflammation and immune mechanisms. Their functions include inducing the differentation and proliferation of B and T cells, directing B cells to switch the type of antibody they produce, activating NK cells, and inducing fever.
- o Tumor necrosis factor (alpha and beta) is released by macrophages and T cells, often in response to bacterial infections. TNF induces inflammation and fever, attracts neutrophils and monocytes, and activates macrophages.

Goals of immune system activities

The immune system components described above interact in a number of ways to combat infectious agents. These interactions are directed toward accomplishing specific objectives related to destroying the infectious agent or limiting its virulence. These objectives include:

- Prevent cellular invasion and intracellular reproduction. A number of immune forces combat intracellular pathogens by preventing them from adhering to the cell, entering the cell, or reproducing in the cell. For example, IgA is important in fighting infections of the respiratory, gastrointestinal and urinogenital tracts (e.g., rhinovirus infections) because it combines with the surface of microorganisms to prevent attachment to epithelial surfaces. Antiviral antibodies have also been found to block entry of viruses into the cell or uncoating once inside the cell. Antibodies produced against *Plasmodium* parasites are thought to protect against both invasion of and growth within the cell. And as mentioned above, interferon binds to cell membranes to prevent the replication of viruses in those cells.
- Neutralize toxins: One of the protective mechanisms shown by antibodies is the neutralization of toxins produced by microbes (e.g., *Clostridium tetani* and *Corynebacterium diphtheriae* toxins). By binding to these toxins, tetanus and diphtheria antibodies interfere with their ability to cause disease.
- Lyse or otherwise damage an extracellular pathogen or infected cell: A number of cells or compounds are capable of inflicting damage on a pathogen or infected cell at close range.
  - o Killer T cells: These cells quickly and efficiently lyse virally-infected cells displaying MHC Class 1 compounds through the activity of the molecule perforin.
  - o NK cells: NK cells lyse cells infected with viruses, particularly those viruses that reduce the MHC Class 1 expression on cells they infect in an apparent attempt to avoid killer T cells (herpesviruses, adenovirus). NK cells also have receptors for IgG and therefore can recognize and destroy target cells covered with IgG through a process called antibody-dependent cell mediated cytotoxicity (ADCC).
  - o Complement membrane attack complex (MAC): Complement molecules are able to lyse virus-infected cells or microorganisms such as Gram-

negative bacteria and enveloped viruses directly through the formation of a membrane attack complex (MAC) that opens holes in cell walls.

- o Eosinophils: These cells are attracted to sites of infection by substances released by mast cells and basophils. They attack helminths by releasing various substances from their granules that damage cell membranes. Antibodies (particularly IgE) assist by attaching to helminth larvae, thereby facilitating their recognition and destruction.

- Phagocytose the pathogen. Neutrophils and macrophages are the two types of professional phagocytes (cells whose main function is to engulf and destroy pathogens and other materials). Their objective is to surround the pathogen, enclose it in a phagosome, fuse the phagosome with a lysosome that contains enzymes, and then release the enzymes to degrade the pathogen. In addition to enclosing and killing the foreign agent, macrophages also present antigen fragments to immune cells. Processes that facilitate phagocytosis include:
  - o Opsonization: Antibodies and complement components, acting together or separately, coat the microorganism to make binding easier. Opsonization is important in controlling *Neisseria meningitidis, Candida*, and encapsulated pneumococcus.
  - o Agglutination: Antibodies will agglutinate smaller microorganisms to reduce the number of infectious units and facilitate their phagocytosis. This mechanism may be helpful in eliminating blood-borne viruses.

- Combine with extracellular organisms to inhibit metabolism or growth. For instance, it appears that certain antibodies that target surface antigens on *Mycoplasma* species are capable of inhibiting the growth of these bacteria.

- Immobilize the pathogen. Inhibiting the motility of an organism may reduce its virulence, for instance by decreasing its ability to adhere to or infect host cells. Antiflagellar antibodies have been identified for bacterial species such as *Pseudomonas aeruginosa.*

- Expel the pathogen. Some factors in the body can act to physically drive out the pathogen. For instance, mast cells responding to the presence of parasitic worms can, by releasing histamine, trigger smooth muscle contractions that help to expel the invaders.

- Make the environment less hospitable. An indirect method of controlling virulence is to change the environmental conditions within the body so that infectious agents are less able to grow and multiply. For example, it is thought that the induction of fever by pyrogenic substances (e.g., interferon and interleukins-1 and -6) helps to inhibit the multiplication of certain viruses. *E. chaffeensis* is controlled through the cytokine-mediated sequestration of iron, a metabolic requirement, and the fungus *Paracoccidioides brasiliensis* appears to be inhibited from transforming from the conidia to the yeast form by the release of nitric oxide and the restriction of iron. Other environmental variables that can be controlled to reduce virulence include pH and oxygen tension.

Immune response avoidance and interference

Just as there are a large number of immune defenses that can be brought to bear against an infection, pathogens likewise exhibit a large number of mechanisms to avoid

these defenses. Each of these mechanisms is directed at letting the infectious agent take hold, spread, and multiply before the infection can be brought under control.

Take advantage of immune system weaknesses
Some diseases are the result not of a particularly effective or novel virulence factor but instead are due to the absence of a strong immune response. For instance, immature fetal immune response can lead to prenatal infections that are much more severe than postnatal infections, as is the case in rubella (German measles). Some infections are enabled by other conditions that have a general immunosuppressive effect, decreasing T-cell and B-cell responses and thereby providing opportunities for unrelated infections to establish themselves. Diseases that are thought to be immunosuppressive include Epstein-Barr and malaria. In some cases diseases depress immunity to selected pathogens, often through unknown mechanisms (antigenic competition has been proposed as one possible mechanism). In others the immune function is disabled in a more general way. HIV, for example, by infecting Th cells and macrophages, enables a wide range of infections, often by opportunistic pathogens that normally would be controlled with ease. Because these unrelated infections often prove to be fatal, this immunosuppressive strategy in one sense can be thought to limit the overall effectiveness of HIV.

Actively weaken specific immune responses
Some infectious agents are capable of weakening certain components of the immune system for their own specific benefit (as opposed to general immunosuppression, described above, which primarily benefits other microorganisms). This capability may be responsible for the establishment of infections such as cryptococcosis or some forms of leishmaniasis. One mechanism through which this might happen is the induction of immune tolerance to an antigen. It has been hypothesized that high levels of circulating antigen may cause immune cells to become desensitized to that antigen, resulting in anergy, or the loss of the ability to effectively respond. Another mechanism may be the targeting of immune cells specific to a pathogen. The suggestion has been made, for example, that HIV is capable of restricting the proliferation of HIV-specific CD4+ T cells, thereby inhibiting the immune response to HIV.

Evade the immune response
One set of strategies for dealing with the immune system involves limiting the availability of addressable targets presented to immune forces. These strategies include:
- Staying inside infected cells and allowing little antigen to form on the cell surface. For instance, Epstein-Barr is able to persist in lymphocytes in part because a potential antigen (EBNA-1) resists proteolysis and thus presentation on the surface of the cell. Limiting activity within a cell also minimizes the risk of recognition. Malaria parasites inhabiting liver cells (hypnozoites) are capable of remaining in a dormant state for up to two years, thereby avoiding detection.
- Avoiding extracellular fluids. Pathogens that can spread directly from one cell to the next avoid the risk of attracting antibodies that are present in the extracellular fluid. For instance, herpes simplex virus spreads directly from cell to cell in the peripheral nervous system, as does *Shigella* in the colon. Infections can also spread directly from cell to cell during cell division, such as during fetal development.

- Decreasing the level of MHC on infected cells. MHC class I and II proteins bound to viral antigens activate T cells; therefore, decreasing the amount of MHC available to bind to antigens limits the T cell response against a virus. One way to do this is to inhibit the expression of MHC proteins on the cell surface. Several herpesviruses including cytomegalovirus and varicella-zoster virus are capable of this, as are adenoviruses.   One mechanism is to prevent manufactured MHC proteins from reaching the surface of the cell (adenovirus type 2 produces a protein that binds MHC to the endoplasmic reticulum; varicella-zoster virus also impairs the transport of MHC molecules to the cell surface). Another mechanism, thought to be exhibited by human cytomegalovirus and other viruses, is to inhibit the transcription of MHC proteins by interfering with cytokine signal transduction in the infected cell.

Inhabit sites that are unreachable by immune forces
There are a few sites in the body that are accessible to infectious agents but difficult for T cells or antibodies to reach. Outward-facing cells in tissues such as salivary glands are difficult for cells to reach and thus can harbor viruses such as herpes simplex virus and cytomegalovirus. The epidermis, hair follicles and nails are examples of sites that are distant from the immune forces that would otherwise be able control pathogens such as wart viruses.

Divert the immune response
Some pathogens are able to direct the immune system into mounting inappropriate, ineffective or even harmful immune responses instead of effective responses directed specifically at the invader. Some infections induce the production of antibodies that have low avidities and therefore are ineffective at controlling the disease. Others induce production of antibodies that are either nonspecific or directed against host tissues (as in African trypanosomiasis and Epstein-Barr infections). Still others may be capable of provoking a type of immune response that is less effective than others, for instance a vigorous antibody response instead of a more helpful T-cell response. *Mycobacterium leprae* may even induce the proliferation of T suppressor cells, thus limiting the cellular immune response.

Resistance to immune forces
Some infectious agents do not prevent an immune response from being mounted, but rather are able to withstand the response or interfere with it so that infection can proceed. Fortification, prevention of attachment, disabling of defenses, deception, and resistance of phagocytosis are all various forms of this approach.
- Fortification. Encystment is a method by which microorganisms can protect themselves from host defenses. Some protozoa can form cysts around themselves inside a cell (for instance, *Toxoplasma gondii* encysts itself inside macrophages). Helminth parasites (e.g., tapeworms) also form cysts that limit their exposure to antibodies or immune cells.
- Prevention of attachment. Since direct contact with the pathogen is a requirement of most defense strategies, it stands to reason that inhibiting attachment would help make a pathogen difficult to control. There are many ways in which this can be accomplished:
  - Some strains of staphylococci inhibit access and phagocytosis by producing a coagulase that induces the protective deposition of host fibrin

around the bacteria; the bacteria also excrete protein A which attaches to the Fc portion of antibodies, providing protection from opsonization.

- o Virulent strains of Gram-negative bacteria exhibit O antigens on polysaccharide projections, far away from the cell wall; this distance prevents lysis of the cell wall by complement.
- o *H. influenzae, Streptococcus pneumoniae, E. coli* K1, and *N. meningitidis* surround themselves with carbohydrate capsules that protect them from complement lysis, antibody deposition and phagocytosis.
- o Sialylation of a terminal site on an LPS surface component in gonococci and meningococci prevents lysis by complement, lets the bacteria avoid contact with and ingestion by phagocytes, and prevents LPS from reacting with specific antibodies.
- o The negative surface charge of the cell walls of *Staphylococcus aureus* and *Salmonella enterica*, among others, has been reduced in some strains to repel and prevent the attachment of cationic antimicrobial peptides (CAMPs).

- Disabling of defenses. Another approach used by some pathogens is to synthesize products that defeat important components of the immune response.
   - o Some bacteria manufacture a protease that cleaves IgA1 antibodies (e.g., gonococcus and *Streptococcus pneumoniae*), thereby presumably lessening the effectiveness of the antibody response.
   - o Interference with the complement system takes many forms. *Pseudomonas aeruginosa* produces an enzyme that inactivates C3b and C5a; K antigens on *E. coli* may disrupt the alternative pathway; some viruses (e.g., poxviruses and herpesviruses) encode proteins that inactivate the complement system; and the hyaluronate capsule of some Group A streptococci may provide a physical barrier between C3b and its corresponding receptor on phagocytes.
   - o Viruses demonstrate several different methods of interfering with the activity of interferon and its agents. For example, vaccinia virus produces a receptor that binds to interferon; adenoviruses disrupt signaling induced by interferon; and the hepatitis C virus inactivates PKR, an enzyme induced by interferon that blocks viral protein synthesis.
   - o Some microorganisms secrete substances that inhibit the chemotaxis of phagocytic cells. For example, streptococcal streptolysin O and toxins produced by *S. aureus* repel neutrophils.

- Mimicry and deception. The synthesis and presentation of molecules that resemble normal human counterparts can be an effective method of evading host defenses or stimulating a desired activity.
   - o Viruses are capable of synthesizing proteins that mimic a wide variety of host proteins, including cytokines, chemokines, and their receptors, antiapoptotic factors, cell cycle proteins, and complement regulatory proteins. For example, the Epstein-Barr virus can transform infected cells into tumor cells by expressing viral proteins that mimic activated tumor necrosis factor receptors, thus stimulating cell growth and preventing apoptosis. Also, cytomegalovirus encodes a MHC class I-like protein which migrates to the surface of infected cells to keep NK cells away.
   - o Bacteria may bind host proteins (e.g., fibronectin and collagen) or present molecules that mimic host molecules in order to avoid surveillance. For

example, the Lewis blood group antigens which are expressed by *H. pylori* have been proposed to serve as camouflage since these antigens are also expressed by human gastric epithelial cells.

- Resistance to phagocytosis. Phagocytosis is an important defense mechanism for eliminating microorganisms, particularly bacteria. Most pathogens are sure to encounter neutrophils and macrophages at one time or another; therefore it is of great advantage to be able to survive or even benefit from the encounter. Two strategies for resisting phagocytosis include:
  - o Survive and grow within the phagocytic cell: Certain pathogens are well-suited to the environment found within phagocytic cells and thus can remain and multiply there. This often necessitates defeating defense mechanisms within the cell. For instance, *Leishmania* inhibits cellular signaling pathways in its macrophage host which otherwise would lead to full activation of macrophage and induction of a strong microbicidal response. *E. chaffeensis*, among others, is able to live successfully within phagosomes because it inhibits the fusion of these vacuoles with destructive lysosomes. *Mycobacterium tuberculosis* also inhibits lysosomal fusion, possibly by retaining a protein in the phagosomal membrane whose absence would indicate that fusion should take place. Some species, such as *Mycobacterium leprae*, are even able to survive lysosomal fusion. Other pathogens, such as *Rickettsiae* and *Listeria monocytogenes*, are able to escape the phagosome and live and multiply in the cytoplasm.
  - o Kill the phagocyte: Some infectious agents are able to kill phagocytic cells using extracellular means. For example, streptolysin produced by virulent streptococci binds to the membranes of phagocytic cells in such a way as to make the lysosomes inside the cells release their contents into the cytoplasm. *Candida albicans* can kill cells after ingestion through its growth mechanism – it grows germ tubes which puncture the membrane of the cell, causing it to die.

Antigenic variation
One other way in which infectious agents avoid host defenses is to present a moving target by continually varying the antigens they present to the immune system. This can happen both at the individual level (a given infection can be marked by repeated antigenic variations) and at the population level (different strains of a pathogen can affect a population at various times). The influenza virus is an example of a pathogen that exhibits population-level antigenic variation. These viruses evolve not only through viral mutations such as point mutations, deletions, and insertions, but also through recombination in hosts infected with two strains (e.g., a human and an animal strain). Pathogens that exhibit antigenic variation at the individual level include *Trypanosoma brucei*, whose surface coat changes as a result of spontaneous gene mutations after each new humoral assault, *Neisseria gonorrhoeae*, whose ability to adhere to a variety of surfaces is due to continually altered genetic expression for pili and outer membrane proteins, and HIV, whose lack of effective replication proofreading mechanisms leads to its high rate of antigenic variation.

Phenotypic characteristics

Infectious diseases are generally characterized by wide variations in expressivity and penetrance within a population. A variety of factors determine whether a person will be exposed to an infectious agent and if so, whether the exposure will be controlled immediately or lead to disease. Similarly, once a person contracts a disease, multiple factors influence the nature and extent of the symptoms as well as the course of the disease and outcome experienced by that person. These influential factors can be thought of as pertaining to a population, to an individual, or to the pathogen itself.

Population factors

Some diseases (rhinovirus colds, for instance) are worldwide in spread and affect all races and socioeconomic classes. However, many diseases are found to be much more prevalent in certain populations than in others. Factors that induce a population to be more susceptible to a given disease include:

- Geography. In order for a pathogen to thrive, environmental conditions must be suitable for it and/or its host(s). Therefore, when an agent or its host has specific habitat requirements, the disease will be closely associated with geographical areas that meet those requirements. For example, *Histoplasma capsulatum*, a fungus associated with bird and bat guano, is found throughout the United States, but is especially prevalent in Mississippi and Ohio River valleys and is also frequently encountered in caves. Blackflies, the animal vector of the filarial nematode *Onchocerca volvulus*, breed in oxygen-rich water and therefore the disease onchocerciasis tends to be more prevalent in areas around rapid streams.
- Living conditions. Certain conditions such as crowding and poor sanitation greatly facilitate the spread of diseases such as shigellosis and amebiasis through increased contact and contamination, such as fecal contamination of drinking water.
- Genetics. Susceptibility to a disease may be mediated by genetic factors, so that people who acquire specific alleles are more or less likely to contract the disease than others. Malaria is a classic example of a disease in which susceptibility is mediated by genetic factors. One such malarial susceptibility factor is sickle cell trait, which provides protection against *Plasmodium falciparum*. Also, the absence of Duffy bloodgroup antigens (a factor required for cell invasion by *Plasmodium vivax*) in certain African populations leads to the absence of *P. vivax* infections in these areas.

Individual factors

A variety of factors influence whether an individual exposed to an infectious agent will develop the disease, and what will happen during the course of his/her disease. These factors include:

- Age. Immunological and physiological changes that take place as people age have a strong effect on the host's response to a pathogen. Young children whose immune systems are not fully developed and older people whose immune systems have weakened with age both have increased susceptibility to infections. Physiological immaturity or age may also influence the degree of damage inflicted by an organism. For example, for physiological reasons, infants

and the elderly are both more susceptible to respiratory infections. Conversely, many viruses (varicella-zoster and poliovirus, to name two) affect adults more severely than children for reasons not yet known. Social behaviors associated with different age groups also play a role in the spread of disease. For example, contact between children is often responsible for the transmission of "typical" childhood diseases, just as sexual contact in adolescence and adulthood leads to the spread of sexually-transmitted diseases.

- Occupation/environment. Features of a person's working or living environment can greatly influence their chances of contracting a disease. Some diseases primarily afflict people engaged in a particular occupation – for instance, Q fever generally affects people working with livestock because the most common reservoirs for *Coxiella burnetii* are cattle, sheep and goats. Other diseases may be associated with types of environment. For example, shigellosis, which is spread through the fecal-oral route, is often found in institutional environments such as day care centers and nursing homes.

- Gender. Although most infectious diseases appear to affect males and females equally, there are a few diseases that display gender variations in terms of susceptibility and severity. For example, whooping cough and infectious hepatitis have a slightly more severe effect on women, while *P. brasiliensis* affects more men than women by a ratio of up to 78:1. Pregnancy can also increase the severity of certain diseases.   Differences in hormone levels may account for the varying effects exhibited by these diseases in men versus women. This appears to be the case in *P. brasiliensis;* administering female hormones to men infected with this fungus confers resistance by blocking the conidia- or mycelium-to-yeast transition.

- Health condition/medical history. An individual's past and present state of health may include factors that will affect the severity and outcome of an infection. Some infections take advantage of existing health conditions that facilitate the adherence and spread of microorganisms. Two such diseases are infective endocarditis, in which oral bacteria inhabiting the bloodstream are able to settle on abnormal heart valves, and staphylococcal osteomyelitis, in which trauma to the growing ends of bones likewise promotes the colonization of bloodborne bacteria. Individuals with compromised immune systems are susceptible to severe diseases caused by organisms that normally would be harmless or at least more easily dispatched. Cryptococcosis is often life-threatening in people with AIDS but usually symptomless in people with normal immune systems. Conversely, it has been suggested that the presence of certain organisms can actually be protective against infections by other organisms through the modulation of immune system forces. For example, one theory states that the down-regulation of Th1 responses by intestinal helminths may protect against Crohn's disease. Nutritional status and stress are two other health factors thought to affect host susceptibility to disease or the course of a disease. For example, stress is thought to be one factor that can trigger recurrent reactivations of latent herpesvirus infections. Finally, being vaccinated against a certain infectious disease will certainly decrease a person's overall chances of contracting that disease.

Pathogen-related factors

As detailed in the above sections, factors related to the pathogen itself greatly determine how it may enter the body, what tissues it targets, how its infection of the body leads to disease, and how host defenses organize to defeat it. Even within a particular type of pathogen, slight genetic differences can exert a large influence on an organism's virulence and thus on the phenotype it brings about. For example, there are virulent as well as avirulent strains of *Escherichia coli*, and the virulent strains bring about different forms of disease depending on the specific virulence factors each possesses.

Phenotypic characteristics that derive from pathogen-related factors include nature of symptoms, duration, recurrence, and infectivity.

- Nature of symptoms. Factors that influence the type of symptoms that will be experienced during the course of a disease include the tissue targeted by the pathogen (a pathogen that targets the pharynx will likely cause a sore throat), and what types of inflammatory response is induced (e.g., pathogens that induce the release of pyrogenic cytokines will cause fever). The ability of a pathogen or its toxins to spread via the bloodstream or lymphatic system will also affect whether the disease is localized to one site or spreads to many sites over time. For instance, measles virus can spread throughout the body to cause a variety of symptoms and conditions including rash, runny nose, conjunctivitis, pneumonia and encephalitis. The severity of symptoms also varies greatly, ranging from those that are at most annoying (e.g., cutaneous warts caused by human papillomaviruses) to those that are life-threatening or fatal (the central nervous system destruction seen in Creutzfeldt-Jakob disease).

- Duration of disease. The duration of an infectious disease can range from days or weeks to years, depending on how quickly and how effectively the pathogen can be brought under control. For example, in *Salmonella* poisoning and influenza the symptoms generally last only a week or two; in other diseases such as leprosy, the symptoms are continually present and may never resolve. In many diseases, recovery is accompanied by the pathogen being fully cleared from the host. In others, the pathogen persists in the body, either continuing to cause disease (as in leprosy) or maintaining a subclinical presence. Naturally, in order to persist in a host, the pathogen must be able to successfully contend with or evade immune system defenses.

- Recurrence of disease. Some diseases associated with persistent pathogens have a relapsing/remitting nature, with symptoms that periodically recur. Often the pathogen involved is able to maintain a presence in a latent or dormant state for a period of time, without engaging in activities that would attract an immune response. Later reactivation of the pathogen gives rise to a new episode of disease. The herpes simplex virus maintains a latent infection in sensory neurons, but when reactivated by stress, sunlight or other factors, travels down a sensory nerve to cause a lesion. Shingles arise through a similar mechanism: the varicella-zoster virus that causes an initial chickenpox infection persists afterward in sensory neurons, controlled by cellular immune forces. As the host ages and the immune system weakens, the virus may multiply and spread down a sensory nerve to cause shingles. Recurrent infections may also be caused by antigenic variation. For instance, the relapsing febrile episodes caused by *Borrelia recurrentis* are due to a succession of new antigenic variants, each emerging as the previous variant comes under control of the immune system.

- Infectivity. Some pathogens are extremely well-adapted to entering and establishing themselves within the host to the point where they can cause disease, even when starting with a very small presence. Other pathogens are not as infective and therefore a large number of them is required to have entered a host in order for clinical manifestations to be seen. For instance, *Shigella* and *Cryptosporidium* have very low infectious doses (in the tens or hundreds of infectious units), whereas the infectious dose for *Vibrio cholerae* may be in the millions. Infectivity depends on a number of factors, including the exact strain of the pathogen in question, the transmission vector (e.g., water-borne cholera requires a higher dose than food-borne cholera), the route of entry into the host, and the overall health of the host, to name a few.