Pathogenesis - The Microbes' Side

A pervasive motif in pathogenic microbiology that has gained currency only recently is that microorganisms do not simply grow in the host but also APPROPRIATE MANY OF THE HOST FUNCTIONS FOR THEIR OWN PURPOSES. A whole new field, known as CELLULAR MICROBIOLOGY has arisen as the result of these studies. Research in this field is helping to elucidate basic mechanisms involved in eukaryotic cell biology.

This is old hat with the viruses because they can only replicate by appropriating the cell’s machinery. Some viruses possess a few enzymes for nucleic acid replication, but none of them have the whole set necessary for growth and none of them can make a source of energy. Most pathogenic bacteria, on the other hand, can grow on agar plates. For a long time, it was thought that bacteria use the body as a sort of giant Petri dish and that they simply grow in one or another part of it. There was no reason to believe that bacteria in the laboratory and inside the host do very different things. This notion has been turned on its head as the result of newer research. Increasingly, it turns out that bacteria are programmed to do very different things when they are inside the body or in the laboratory. Obviously, some of the housekeeping genes involved in central metabolism and macromolecular biosynthesis will be equally turned on in both cases. However, analysis of gene expression has revealed that a large number of genes are switched on only when bacteria invade a host.

Presented here are four examples of infectious diseases. Each one serves to illustrate a number of features, some characteristic of the particular agent, others common to several of them. It may be helpful to think of these diseases in terms of the general steps of pathogenesis (encounter, entry, establishment, damage). In particular, to what extent is the agent or the host responsible for the damage? To what degree does the agent usurp host functions for its survival and replication? What is the interplay between the host defenses and the way the agent tries to subvert them?

Case Report - Tetanus, a relatively “simple” infectious disease

A 22-year-old farm worker came to the doctor’s office complaining of pain in his jaw and inability to open his mouth fully for last three days. Ten days before, he had pushed inadvertently against a rusty nail sticking out from a plank in a horse corral. The nail had penetrated deeply through the skin, and although the wound hurt and bled, he had not sought medical attention. He had received his tetanus shots as a child and his last booster was more than 10 years ago.

The patient was told about the possibility of tetanus and received two doses of human antitetanus immunoglobulins intramuscularly into each buttock, as well as an antibiotic.
Over a period of several weeks, his symptoms gradually subsided. His wound stopped draining by the next day, and the redness around the wound was fading.

Tetanus is a disease caused by the toxin produced by Clostridium tetani. This organism is very common in soil and feces. It forms spores, which explains its long ability to persist for long periods in the environment. C. tetani release a toxin that interferes with the inhibitory neurotransmitters, causing the extraordinary tightening of muscles (spastic paralysis), including what this patient was experiencing. Left untreated, tetanus may result in death by asphyxiation. This patient did well, possibly because had some residual protection from his previous tetanus shots. The toxin of the agent of botulism, C. botulinum, may also cause asphyxiation, but doe so by inducing the relaxation of muscle (flaccid paralysis). For this reason, botulinum toxin (Botox®) is used for cosmetic and occasionally therapeutic purposes.

C. tetani is related to C. botulinum, and shares many attributes with it. Neither organism has much invasive power and only penetrates via breaks in the body’s integrity, such as those caused by wounds and contaminated syringes. Although one thinks of botulism as being caused by eating the contents of improperly sterilized cans, this disease can also be acquired from wound contamination. A rare disease in the past, wound botulism has been on the rise among infected drug users. As the result of vaccination, tetanus is relatively infrequent in the United States but there are still some 50 cases per year. It is quite common in countries with poor hygiene and lack of vaccination.

What must take place for tetanus or botulism to become established? Once inside tissues, these organisms cause local inflammation at the site of entry. Tetanus and botulism bacilli do not survive for very long in tissues and are usually cleared from the site in a matter of days. One reason is that these organisms are strict anaerobes and do not grow in the presence of oxygen. Severe damage, then, is not a local phenomenon but takes place at a distant site. In fact, the local wound may be so slight as not to be noticed. The reason why relatively few bacteria can cause such a potentially devastating diseases is that the toxins are enormously powerful and works at minute concentrations. Botulism and diphtheria are among the most powerful poisons known (one gram of toxin could kill some 10 million people!) It has been joked that purified botulism toxin is a white powder of unknown taste.

To understanding what goes on in a patient with tetanus, one must focus on a several issues: where does the organism reside in the environment and how is it encountered, how does it enter the body, how does it survive the local defenses (at least for a short time), how is the toxin spread to the target tissue (in this case the nervous system), and how the toxin acts once it gets there. Tetanus is certainly not simple, but may appear so in comparison with other infectious diseases.
Case Report – An outbreak of hemorrhagic colitis – a complicated infection caused by E. coli strain O157-H7

In 1999, a food borne outbreak took place among people attending the Washington County Fair in New York State. The causative organism was found to be a strain of Escherichia coli called 0157:H7. The source of the organisms may have been from a well on the fairgrounds that was probably contaminated with cow manure. In other outbreaks, the source of the organism has been traced to undercooked hamburger meat. In this one, a 3-year old girl and a 79-year-old man died from complications of the infection. Hundreds of others became ill with a bloody diarrhea, a condition known as HEMORRHAGIC COLITIS. Seventy-one were people had to be hospitalized. Of these, 14 developed a severe complication of E. coli O157:H7 infection (the hemolytic uremic syndrome) that can lead to kidney failure.

E. coli O157:H7 is one of a large number of strains in this species. In fact, the limits of the species Escherichia coli are very broad, and include a large number of pathogenic varieties. Most of them cause intestinal disease, but some are frequent causes of urinary tract infections, and a few invade deep tissues. On the other end of the spectrum is the K12 strain, an old laboratory workhorse that has not been shown to colonize anyone, leave alone cause disease, among the innumerable workers who come in contact with it or volunteers who were fed cultures of this strain. Although the genome of all E. coli strains is similar, many of the pathogenic ones carry upward of 20% more DNA. It seems probable that these extra genes were acquired by lateral transmission from other bacteria.

The E. coli strain that caused the above outbreak normally resides in the intestine of cattle, where it causes little if any perceptible disease. Animals colonized with this organism become CARRIERS. Like many other infectious agents, this organism causes serious disease in one host and little if any in another. Humans are ACCIDENTAL HOSTS of this organism, which would survive quite well if it never infected people. Diseases acquired from animals are called ZOO NOSES (there are diseases that animals get from people, but the term “anthroponoses” has not gained currency).

Once ingested, the organisms make their way to the large intestine, where they cause inflammation (hence “colitis”). They are relatively acid resistant, which explains how they survive the trip through the stomach. The organisms do not invade the intestinal epithelium and are confined to its surface and must adhere to it in order not to be swept away by liquid currents. Soon, the adhering bacteria destroy the villi of the intestinal epithelial cells and create a lesion that impairs intestinal function and causes severe diarrhea. When the lesion becomes deep enough, it breaks through the layer underneath the epithelium, the lamina propria, and affects the underlying blood vessels. This results in hemorrhage and bloody stools. It is thought that the profuse bleeding is brought about by INFLAMMATORY CYTOKINES that are elicited by toxins made by the organisms. Complications of this disease are infrequent and are due to the outpouring of a toxin into the blood circulation, leading to damage of small blood vessels in the kidneys, brain, and other organs. This can cause death.

Toxins made by these organisms are not secreted into the medium but, surprisingly, are INTRODUCED DIRECTLY into the host cell. Contrast this with the tetanus bacillus: it secretes a toxin that is spread all over the body. Here, this dilution effect does not matter because tetanus toxin is so potent that only a few molecules need to reach their target. E. coli uses a much more sparing mechanism: there is no waste here because all the protein molecules reach sensitive cells. The introduction of bacterial proteins into host cells superficially resembles injection via a syringe. Sticking out from the bacteria is a microscopic apparatus that makes contact with the host cell surface. However, this analogy is
misleading because there is no plunger. This machinery bears the nondescript name of **Type III secretion apparatus**. This apparatus consists of several structural proteins that are homologous to proteins of bacterial flagella, suggesting that these both these nanomachines may have had a common origin. The Type III secretion apparatus is **made on demand only**. The stimuli sensed include temperature, ionic conditions, and the surface of sensitive cells, which are properties of the environment in the body not readily duplicated in the laboratory.

Toxins delivered to the host cell by Type III secretion have several functions: they alter the cell’s cytoskeleton, leading to morphological changes that favor attachment of the bacteria, and they lead to the destruction of the intestinal cell microvilli. In additions to the toxins delivered into host cells, this secretion apparatus is also used to introduce a receptor molecule needed for bacterial adherence. The first step in binding of the organisms to the cell surface is relatively weak and, to ensure that the bacteria are not dislodged, must be followed by stronger adherence. That bacteria provide a receptor for their own binding may seem unusual. The organisms can be expected to have a ligand, but the host cell normally provides the receptor. However, these *E. coli* use Type III secretion to make a receptor themselves. One of the proteins injected into the cell is called Tir. In the cytosol, Tir becomes phosphorylated and in this modified form has affinity for the host cell membrane and inserts into it. On the membrane, Tir acts as a strong receptor for the bacteria, which leads to tight binding. In this manner, the bacteria use the host’s phosphorylating system to convert one of their own proteins into an effective receptor. This is another example of how microorganisms appropriate cell functions for their own use.

Many bacterial species, not just *E. coli*, use Type III secretion to introduce proteins directly into cells. This mechanism is not uncommon among human and plant pathogens, suggesting that it may have evolved once and that later it may have been transferred laterally between species. This notion is reinforced by the fact that the genes for Type III secretion are often clustered in contiguous regions of the chromosome or plasmids. Such clusters are called **pathogenicity islands**, and may be distinguishable from the rest of the chromosome (for example, by their G+C ratio), as if they had been acquired recently. It seems reasonable that such a sophisticated process as Type III secretion should be used by many pathogenic species.

Other bacteria also make use of the **modification of their proteins in the host cell cytosol**, which has helped explain long mysteries. For example, until recently, there was not a good explanation for how salmonella cause food poisoning. Other bacteria that induce diarrhea, e.g., the cholera bacilli, produce a toxin that can be easily found in culture filtrates or in the contents of a patient’s intestine. Despite many attempts, no such soluble toxin had ever been found for salmonellas. It turns out that, as in the case of *E. coli*, a salmonella protein becomes modified in the host cell cytosol. Here, the modification consists of the enzymatic addition of an NAD-ribosyl group to *actin*. In this modified state, the activity of actin as part of the host cell’s cytoskeleton is altered. It is thought that this leads to internalization of the bacteria into intestinal cells, which are normally not very adept at phagocytosis. Other bacteria make toxins with the same enzymatic activity, but in most cases, the target proteins are different. In the case of diphtheria bacilli and pseudomonas, the affected protein is required for host cell protein synthesis. In cholera, the target protein is one that regulates the level of cyclic AMP required for the proper ionic balance of cells. When this protein does not work properly, there is a heavy outpouring of water that results in the copious liquid diarrhea characteristic of this disease.
Case Report – Tuberculosis, a disease caused mainly by the host response

On a snowy evening, a 32 year old man living on the streets came to a Walk-In Clinic in Boston complaining of a cough he had for several months, fever, and night sweats. He appeared slightly drunk, chronically malnourished, and had a temperature of 102.6° F. Examination of his chest revealed “rales,” crackly lung noises that indicate fluid in the air sacs and are suggestive of pneumonia. After getting a chest X-ray and depositing a sputum sample, he left abruptly and spent the night in an abandoned building he often shared with several friends. Laboratory examination revealed the presence of acid fast bacilli in the sputum, consistent with the tubercle bacillus, Mycobacterium tuberculosis. The X-rays added further credence to the diagnosis of tuberculosis. The patient was HIV negative. When he returned to the clinic four months later, he was given a cocktail of several antibiotics with the stern and explicit admonition that it was essential that he not skip a single dose. This treatment was to last for 9 months. The personnel at the clinic doubted that the patient would comply with this regimen.

Tuberculosis (TB) conjures up the image of a severe lung disease that can be fatal unless treated. Book lovers will be reminded of Thomas Mann’s “Magic Mountain,” and opera fans of the dying heroines in La Traviata and La Bohème (“Che gelida manina….” [“What an icy little hand….“]) . Actually, TB is not a single disease, but one that has many manifestations depending on previous exposure, nutrition, and other health factors. Although relatively rare now in developed countries, worldwide TB is still the leading cause of death from a single infectious disease. It was the cause of the “White Plague” of the 17th and 18th centuries in Europe (not to be confused with the Black Plague, caused by the plague bacillus, Yersinia pestis). During that period nearly 100 percent of the European population was infected, and 25 percent of all adult deaths were due to TB. In the U. S., the number of TB cases has dropped almost every year since records were kept, but since 1985 the number of cases rose, to fall again beginning around 1999. The rise has been largely attributed to the emergence of HIV infections and to the increase in the number of homeless persons (as in the case described). The later fall may be attributed in part to the more successful use of anti-HIV drugs. In recent times, multidrug resistance has emerged among strains of M. tuberculosis, making them particularly threatening. A big reason for drug resistance is non-compliance with long term antibiotic treatment, which leads to the selection of drug resistant mutants. Few diseases have as large a social component as TB.

Pulmonary TB typically has two stages, which is a crucial point to understand the disease. In the first or PRIMARY stage, exposure to the tubercle bacilli, usually by inhalation, leads to a mild, self-limiting disease that may be totally imperceptible or as mild as a cold. In most healthy people, there are no more symptoms. In people whose defense mechanisms are lowered, noticeably or imperceptibly, a much more serious disease, SECONDARY TB, emerges later. The time span between the first and the second stages ranges from months to many years. Secondary TB produces the disease that is classically associated with the deadly image of tuberculosis. Between the two stages, the tubercle bacilli LIE DORMANT WITHIN MACROPHAGES and are little noticed by the host.

Tubercle bacilli have several distinctive characteristics that help understand the disease process. They are ACID FAST, meaning that they are quite resistant to acids and other chemicals. Acid fastness is rare among bacteria and confined to a small group only. These organisms have a capsule of waxes that surrounds them and makes them impermeable to many polar molecules. These include the common germicides used to “swab the decks” in hospitals. Mycobacteria are singularly preoccupied with lipid metabolism, both synthesis
and utilization. A disproportionately large number of genes are dedicated to their metabolism. The mycobacterial capsule contains unique waxes called MYCOLIC ACIDS, long chain branched fatty acids about 80 carbons long. Some genes involved in the synthesis and export of lipids are essential for both the primary infection and the subsequent persistence of the organisms. Others genes, such as one that adds cyclopropane residues to the mycolic acids, are not needed for growth during the acute phase. However, mutants deficient in the gene coding for this function cannot cause a persistent long-term infection.

The wax capsule makes tubercle bacilli resistant to drying. In advanced stages of pulmonary TB, patient cough up huge numbers of the organisms which can then persist in the air in aerosols or as dust particles. These two properties, causing a pulmonary disease and being resistant to drying, conspire to increase the chances that these organisms will be transmitted to other persons. Another relevant property of tubercle bacilli is that they grow very slowly, doubling about once every 14 hours. This delays laboratory diagnosis by cultivation because it can take several weeks to see a colony on agar. Once grown, however, the colonies of tubercle bacilli are distinctive—they appear as yellowish lumps of wax on the agar.

The number of tubercle bacilli required to cause create an aerosol is quite high. However, when people are crowded together, as in poor housing or jails, the likelihood of contracting the organisms increases. It seems plausible that the patient described inhaled a sufficient inoculum from the persons with whom he shared refuge and that he could become a source of further spread of the organisms.

What makes people sick with TB? Tubercle bacilli do not make toxins or other products that damage cells directly. Their slow growth signals no intent to rapidly overwhelm the host—they are nearly innocent bystanders. However their very presence is enough to indirectly cause damage to tissues because some of their constituents are noticed by the immune system of the host. In fact, it is the HOST RESPONSE TO THESE ORGANISMS that accounts for most of the symptoms of the disease. Tissue damage is caused by an uncontrolled, progressive, and inflammation that results in severe lesions. It follows that the disease is manifested differently in a “virgin” host and one that has been harboring the organisms.

Primary TB is well controlled in most people. However, the organisms are not cleared and remain viable within macrophages. Contact of a patient with the organisms can be readily demonstrated by the TUBERCULIN TEST. When bacterial antigens, collectively called TUBERCULIN, are introduced into the skin, a cell-mediated immunity reaction is manifested in a person who harbors or has harbored the organisms. A positive test is seen as local reddening and hardening at the site of inoculation in the skin. Many people who have had contact with tubercle bacilli in their youth remain tuberculin positive for years. This attests to the long range survival of the organisms in the body and their ability to provide “booster shots” from within. The ability of the body to keep the organisms under control is, of course, impaired in immunocompromised patients. Here, the disease may rapidly progress to an invasion of many organs and tissues of the body and be fatal.

SECONDARY TB is usually a disease of persons with defects in their immune system, be they relative mild or even unrecognized. Here the balance between microbe and host is tilted in favor of the organisms. The body answers with a vigorous CELL-MEDIATED RESPONSE that damages the nearby tissues. Some of the bacterial chemicals involved are the mycolic acid and muramyl dipeptide, a breakdown product of the cell wall. These two compounds bind to receptors on macrophages, which leads them to release cytokines. One of these, called the TUMOR NECROSIS FACTOR-ALPHA causes severe inflammation. Much of the damage is also caused by the release into tissues of toxic lysosomal components of the macrophages trying to kill the Mycobacterium tuberculosis. The result is
necrosis. When a lesion becomes sufficiently large, it becomes a mass of cheesy-looking material containing few host cell and many bacteria. In the lung, such a lesion may break through into the airways and the contents coughed up, leaving a hole behind. At this stage, the disease progresses rapidly. It was known as the “galloping consumption.”

Given that the immune response is responsible for so much damage, one may ask if it does more bad than good and whether we would be better off without it. Obviously, the immune system is capable of damaging the host, even with fatal consequences. However, in most people TB is a slow disease that only accelerates in its last stages. People with TB can live with it for quite a few years, even without treatment. Contrast this with TB in an AIDS patient. Here, the latent tubercle bacilli rapidly cause a severe, life threatening disease. The choice is clear.

The interaction between humans and the acid fast bacteria does not end here. The organism that causes leprosy is also an acid fast Mycobacterium leprae and shares some features in common with tubercle bacilli. However, it has resisted cultivation to date and can only be studied in a few animals or, more recently, by cloning its genes into surrogate organisms. There are many other acid fast bacteria in waters and soil. Most of them are quite benign and are rarely the cause of disease in healthy people. However, they are quite dangerous in immunocompromised people and are a frequent cause of severe infections in AIDS patients.
Case report  - Infectious Mono – The “Kissing Disease”

A 19-year-old, healthy male football player developed flu like symptoms one week before a homecoming game. He complained to the doctor of a sore throat, low-grade fever, swollen glands, and malaise (feeling ill). A rapid antibody-screening test (costing about $25) was positive. He was told that he probably had infectious mononucleosis and that, to his and his coach’s chagrin, he should not participate in sports for at least one month. A more definitive test (costing about $300) came back positive two days later.

INFECTIOUS MONONUCLEOSIS (“infectious mono”) is most common in people 10 to 35 years old, with its highest incidence in the 15 to 17 years old. Infectious mono is not usually considered a dangerous illness but it may lead to serious complications. An older but quite descriptive name for this disease is “glandular fever,” for the swelling of lymph nodes. It is caused by the EPSTEIN-BARR VIRUS or EBV. The virus is usually transmitted though saliva and mucus—hence its nickname “kissing disease.” The virus can also be spread by sneezing or sharing a drinking glass or straw with an infected person. The incubation period of the disease is not known with certainty, which makes it hard to trace the initial contact. That person may not have had any symptoms because the virus can be carried without signs of illness.

Infectious mono is cause by two DNA viruses, the more common one being EBV, the other CYTOMEGALOVIRUS or CMV. These viruses belong to the family of HERPES VIRUSES, which includes the agents that cause cold sores and chickenpox. About half the people in the US become infected with EBV sometime during their lives without noticeable consequences. Most often, the infection takes place in childhood, where the infections cause either no symptoms or symptoms indistinguishable from other mild illnesses of childhood. By age 40, almost 90 percent of people in the U.S. have antibodies against EBV, suggesting that they have the virus in their systems and are immune to further infection. Persons who do not become infected with EBV until they are in their teens or older are more likely to develop the symptoms of infectious mono. Unusual among human viruses, EBV can PERSISTS FOR LIFE. Thus EBV could fairly be considered to be a member of the normal microbial flora (or biota, if placing a virus within “flora” is stretching the term).

EBV would seem to be a fairly benign agent, causing at worst a fairly mild disease. However, this virus has another face: it can also cause several very serious HUMAN CANCERS, called Burkitt’s lymphoma, Hodgkin’s disease, and nasopharyngeal carcinoma. EBV, then, has three distinct ways of life: it can be

1. a harmless commensal
2. an agent of a mild disease or
3. the cause of serious malignancies.

These characteristics of EBV bring up a number of questions:

• What causes the symptoms in a patient with infectious mono?

• How does EBV withstand the immune response of the host to persist for so long?

• What role does EBV play in cancer? Is it the direct cause of the malignancies?

These questions are related and the answers, alas, convoluted. An understanding of the life style of this virus helps here.

EBV first infects the epithelial cells of mouth and pharynx. The virus then enters the underlying tissues and selectively infects certain cells of the immune system, the B-
LYMPHOCYTES. These cells are primarily involved in antibody production - in contrast to the T-lymphocytes, which play a major role in cell-mediate immunity. Living in cells designed for host defenses may appear to be a dangerous thing for a virus to do. However, many infectious agents have adopted such a strategy, and perversely thrive in such unlikely places. We already considered how the tubercle bacillus resides in macrophages, cells that from a microbial point of view are even more hazardous than B-lymphocytes. In time, EBV-infected B-lymphocytes reenter the lymphatic vessels and spread both to adjacent areas and to the circulation. At any given time, about 20% of the infected people have the virus in their saliva, which accounts for its characteristic form of spread. This also suggests that the virus replicated in privileged sites that are protected from T-lymphocytes.

Why is EBV so specific for B-lymphocytes? The answer is that these cells carry on their surface a specific receptor to which EBV virions binds. Interestingly, this receptor is normally used by the cells to bind proteins of the complement system in order to establish communication between this system and the B-lymphocytes. This is another example of an infectious agent appropriating a normal cell function for its own use.

How does EBV cause Infectious Mono?

Infectious mono occurs in persons that have not been previously infected and have no immunity to EBV. The virus may infect a huge number of B-lymphocytes, up to 20% of all these cells in the body. By itself, this infection has little direct consequences and is not revealed by symptoms. In time, the body begins to make antibodies and to mount a cell mediated immune response. T lymphocytes now come into action, including the subclass called killer cells. Killer T-lymphocytes know how to seek out cells with viral antigens on the surface and destroy them, a one-sided kind of civil war. The outpouring of cellular constituents from the destroyed B-cells causes the disease symptoms, such as fever and inflammation. Here is how: white blood cells, including lymphocytes, have lysosomes (here called “granules”) replete with material that is potentially damaging to tissue, such as hydrolytic enzymes and substances that cause inflammation. Normally, these substances are kept under control as long as the lysosomes are intact. However, when these cells and their lysosomes are broken up, these highly active constituents damage other adjacent cells and tissues. Such events are common in other diseases that result in the death and lysis of white blood cells, such as pus-containing staph or strep infections.

How does EBV persist in the body?

Persistence of any virus (also known as viral latency) requires at least two things: a. that the virus replicates in unison with the cells it infects and b. that the infected cells not be destroyed by the immune system. A commonly used way to ensure concurrent replication is for the viral genome to become integrated into that of the host, much as what happens in lysogenic bacteria. Many viruses, e.g., HIV, herpes, persist by using such a mechanism. EBV, in contrast, resides in the nucleus as an episome, an independently replicating DNA circle. Obviously, EBV must use a special mechanism for viral DNA to be replicated in step with the cell’s chromosomes. This teamwork relies on the interaction of several proteins, some encoded by the virus, some by the host. In addition, cells normally try to eliminate foreign DNA, thus the EBV DNA must somehow be protected from eradication. Certain viral proteins bind to the viral DNA and act in consortium with several cell proteins to make a stable complex that escapes elimination. Normally, these host proteins play a different stabilizing role: they bind to the ends of the linear human chromosomes - the telomeres - and protect them against degradation. EBV, being circular, has no ends but has in its genome sequences similar to those found in the telomeres. Thus,
EBV can appropriate the telomere-binding proteins and turns them to its own use. Instead of working as telomere stabilizers, these proteins now act as viral stabilizers. To prove this, researchers have shown that when the formation of telomeric protein-EBV complexes is inhibited, the latent viral genome becomes unstable and is lost from the cell.

There are several ways for the body to get rid of virus-infected cells and they all must be thwarted for EBV to hang around for the lifetime of the host. One of the reasons for its permanence is that EBV does not reside in all the kinds of B-lymphocytes. Rather, it is found preferentially in a subset of B-lymphocytes called the memory cells. A short digression into immunology: When stimulated with antigens, clones of B-cells are stimulated to proliferate and to make large amounts of a specific antibody. These cells are short lived, and most of them disappear after the antigen is eliminated. If all were eliminated, it would mean that a new encounter with the same agent would require the immune system to start anew. To avoid this, the immune system retains a memory of its past. Some of the B-lymphocytes, the memory cells, are not destroyed but become quiescent in the absence of continual antigenic stimulation. When the body encounters an agent with the same or similar antigenic characteristics, the memory cells rapidly proliferate to mount a rapid and efficient immune response. Immunological memory is the basis why booster shots of vaccines are so efficient and why people infected once become resistant to a second bout of the same infection.

Inside the memory cells, EBV is safe and survives for a long time. These cells, being quiescent, express few viral proteins, thus the virus cannot be "seen" by the immune response. One of the amazing things about EBV is that it targets the part of the immune system that lasts for life – the memory cells – and thus can persist for life.

**How does EBV cause cancer?**

This is not an easy question because the answers may lie on several levels. What is known with assurance is that virus infection of B-lymphocytes stimulates them to become growth-capable cells called lymphoblasts and that, if unrestrained, lymphoblasts grow into lymphomas. In most healthy persons, EBV-induced B-cell proliferation is kept in check by the immune system. Because the immunodeficient state may develop at various times in the life of a person, the onset of cancer may take place many years after the virus is first acquired. Thus, EBV can act as the "mole" of spy stories, remaining hidden until called into action.

Inducing infected B-lymphocytes to proliferate and eventually to make cancers depends on a number of virus-induced proteins. Some of these prevent apoptosis or programmed cell death and thus "immortalize" the cells. This works by a virus-encoded protein activating a host protein that antagonizes apoptosis. Another clever aspect of EBV is that two of the proteins it expresses late in infection precisely mimic a signaling process required to maintain the long term survival of the memory cells.

EBV is also associated with cancers of epithelial cells of the nasopharynx. Why EBV does not readily infect other types of cells? Almost certainly because these cells lack a specific EBV receptor on their surface that is only found in B-lymphocytes and certain epithelial cells. Whatever the reason, this can be counted as good thing.