In the last lecture, we discussed three viruses that enter the body via the respiratory tract: influenza, rhino, and measles. Each of these viruses has an RNA genome, and each uses its own RNA polymerase to make both viral messenger RNA and new viral genomes. This “all RNA” strategy of replication makes these viruses independent of the cellular DNA replication machinery, so epithelial cells which line the airways are fair game for infection, even if they are not proliferating. All three viruses are cytolysis So they leave their host cells dead or dying once they have used them as factories to produce new viruses. The death of infected cells in the respiratory tract and the accompanying inflammation triggers coughs and sneezes, and microdroplets containing virus particles are expelled into the surroundings where they can be inhaled by other individuals to spread the infection.

The rhinovirus genome is protected by a protein capsid which is assembled within the infected cell. In contrast, influenza and measles virus genomes associate with virus-encoded proteins and then pick up an "envelope" made from patches of cell membrane as they exit. Because of the different ways these viruses “dress,” measles and influenza viruses can infect cells deep in the airways where the temperature is 98.6° F. In contrast, the rhinovirus capsid is relatively unstable at this core body temperature, so rhinovirus preferentially infects the upper regions of the respiratory tract where it is cooler. One consequence of this subtle difference in temperature sensitivity is that a rhinovirus infection almost never causes pneumonia.

All three viruses replicate through double-stranded RNA intermediates, and influenza and measles viruses have envelopes. Because of these properties, we would expect all three viruses to induce the expression of large quantities of interferon. This is certainly true of influenza virus, and explains why this virus causes flu-like symptoms. To protect itself from the interferon it produces, influenza virus has evolved mechanisms that reduce the effects of interferon on the cells it infects.

Rhino and measles viruses take a different approach to dealing with the interferon system. Instead of trying to lessen the effect of interferon, both viruses nip the interferon defense in the bud by disrupting interferon production. As a result of this evasion strategy, rhino and measles viruses infections are usually associated with fever and a runny nose, caused by the innate system’s inflammatory reaction to the virus, rather than flu-like symptoms caused by interferon.

Because all three viruses kill the cells they infect, the innate system, which is on the lookout for “excess” cell death, is rapidly activated. In the case of rhinovirus, the innate response is so potent that after a few days, the virus infection is under control. However, during these few days, rhinovirus reproduces quickly and is spread efficiently to new hosts by coughing and sneezing. In fact, rhinovirus’ tactic of “reproduce and surrender” is executed so rapidly that, in most cases, the adaptive immune system is not fully activated, and protective antibodies are not produced.

Measles virus also gets hammered by the innate system in the respiratory tract. To avoid destruction there, this virus infects dendritic cells, and uses a “Trojan horse” strategy to escape the airways and travel to nearby lymph nodes. From these nodes the virus launches a systemic infection in which endothelial and epithelial cells throughout the body are infected. It is this global infection which results
in the typical measles symptoms. Importantly for the virus, this systemic infection brings the virus back to the airways in large numbers, making it possible for measles to infect many epithelial cells and to be spread by coughs and sneezes.

Influenza virus neither surrenders (like rhinovirus) nor escapes (like measles virus). Rather, it “stands and fights” in the respiratory system until, like measles virus, it is eventually subdued by a potent adaptive immune response. However, to evade immunological memory and to expand the pool of infectable humans, influenza virus uses two “bait and switch” strategies. During replication, the error-prone viral polymerase introduces mutations into the influenza genome. The result of this “antigenic drift” is that almost every flu virus is genetically different from every other one. Some of these mutations change the viral hemagglutinin protein, so that neutralizing antibodies, which could bind to the original virus and prevent it from reproducing, now become totally useless in preventing reinfection by the mutant virus. When one of these “escape” mutants enters the population, the result can be an influenza epidemic.

To further expand its list of potential infectees, influenza A virus (but not influenza B or C virus) adds an additional twist to the bait and switch routine. The influenza virus genome is made up of multiple RNA segments, and because influenza A virus can reproduce in both birds and pigs, RNA segments from birds or pigs can be picked up by human type A influenza virus. Sometimes these nonhuman sequences encode hemagglutinin molecules that humans have never seen before, and against which they have no protective antibodies. Consequently, the “antigenic shift,” produced when bird or pig RNA segments are acquired, can lead to devastating, worldwide influenza pandemics.

In addition to the trick of surrendering before immunological memory has fully matured, rhinovirus also uses its error-prone polymerase to create antigenic drift and evade neutralizing antibodies. In contrast, the part of the measles virus’ envelope that is targeted by neutralizing antibodies has a complicated, interlocking structure that cannot “drift” to evade these antibodies without loss of function. As a result, there is only one strain of measles virus, and this strain must be passed in an unbroken chain in which each new infectee has never been infected before.

VIRUSES WE EAT

If the respiratory route of infection represents the easy way in, the digestive tract is most certainly the hard way. The goal of viruses that use this route (the enteric viruses) is to infect epithelial cells that line the walls of the small intestine. To reach these cells, viruses must be able to resist the antiviral defenses present in the saliva, survive exposure to acid pH and digestive enzymes in the stomach, and escape destruction by enzymes that bathe the cells in the intestines these viruses seek to infect. Only a few viruses can do all this. These are their stories.

ROTAVIRUS—an undercover virus

The people who named this virus really got it right. *Rotavirus* is the Latin word for wheel, and that’s just what a rotavirus looks like: a wheel with spokes.

Rotavirus belongs to the reovirus family, and sometimes you hear rotavirus called by its family name. If you classify rotaviruses by the different antibodies that bind to them, there are actually seven different “groups” of rotaviruses. However, group A rotaviruses are the ones that cause most rotavirus-associated diseases in humans, so we’ll limit our discussion to this one group.
Viral Reproduction

Rotavirus is rather unusual in that its genome is made up of eleven segments of double-stranded RNA, protected by not one, not two, but three concentric protein shells (capsids). All viruses face the problem of shedding their protective coats during infection, but for a virus with three coats, you’d expect this to be a particularly difficult maneuver. Rotavirus, however, has figured out some very clever ways to deal with this issue.

The outer capsid of the rotavirus includes two different proteins, VP4 and VP7. The VP7 proteins are the primary building blocks of the outer shell, with VP4 proteins sticking out from this shell like spikes.

Although the details of the mechanism rotavirus uses to enter its target cells are still a little sketchy, it is clear that both VP4 and VP7 play important roles in this process. The current thinking is that a rotavirus is taken into a cell and enclosed in a compartment called an endosome, which is bounded by cellular membranes. It’s worth noting, however, that when biologists don’t understand what’s going on, they usually give the place it happens the suffix “-some”—and this is no exception. In any case, it is believed that within the endosome, the calcium ion concentration starts out being the same as in the environment outside the cell. Then, VP4 or VP7 or both somehow punch holes in the endosome, allowing the calcium ion concentration surrounding the virus to fall (about ten-fold) to the calcium ion concentration of the cell’s cytoplasm. It is this reduction in calcium ion concentration which allows the virus to shed its outer coat. Recent evidence indicates that in the intact virus coat, VP7 is found in “trimers” that consist of three identical VP7 molecules “bundled” together. When the calcium concentration drops, these trimers fall apart, “unbuttoning” the overcoat. One interesting aspect of this uncoating is that neutralizing antibodies bind to the trimeric form of VP7. Virologists speculate that this binding may prevent the virus from shedding its coat by “clamping” the VP7 trimers together. If true, this would be an excellent example of a mechanism by which an antibody can neutralize a virus infection without acting to prevent the virus from binding to its target cell.

Before VP4 can work its magic, it must be cut by a protease to produce smaller, active forms of the protein. If this cleavage doesn’t take place, the rotavirus gets stuck in its coat and is destroyed by the cell it is trying to infect. What’s interesting is that rotavirus’ target cells, the villus epithelial cells that line the intestine, are bathed in proteases. The “day job” of these proteases is to help cut proteins in the food we eat down to size so that they can be taken up by the body. However, one of these enzymes, trypsin, is the very enzyme rotavirus needs to cut VP4. So a rotavirus infection actually requires the function of a digestive enzyme that would destroy most other viruses daring to enter the small intestine! In effect, rotavirus uses what is normally a barrier defense—the proteases present in the intestine—to prepare the virus for entry.

At this point, the virus has removed its “overcoat,” so there are only two coats to go. Now, it might seem that the virus could just go ahead and shed its other two coats, and use its RNA polymerase to copy each strand of its double-stranded RNA. In this way, it could produce both the single-stranded viral mRNA it needs to encode its proteins and the double-stranded RNA required for new genomes. But no. The virus is too smart to do it this way.

In the aqueous environment of the cytoplasm, the two strands of viral RNA would be so tightly bound together that prying them apart to allow the polymerase to work would be very difficult. Double strands of DNA are rather easy to part, DNA-RNA hybrid double strands are more difficult to separate, and the two strands of a relatively long, double-stranded RNA adhere like a tick to a dog. To circumvent this difficulty, rotavirus does something rather ingenious: It uses its RNA polymerase (which is packaged inside the virus particle) to transcribe viral mRNA while the viral genome is still within the protective environment of its double capsid. The single strands of viral mRNA produced by the polymerase are then spit out into the cytoplasm through holes in the double capsid—something like this:
In this illustration I’ve shown only one viral RNA segment. In reality, all eleven segments are transcribed in this way, and many pieces of single-stranded mRNA come sprouting out of the double capsid at the same time. This strategy works well because inside its double capsid, the environment is not aqueous, so the two complementary RNA strands can be separated more easily to allow the polymerase to do its job.

When it comes time to package new viral genomes, the eleven segments of viral mRNA needed for a complete genome are rounded up and cloaked with proteins (including viral polymerase molecules) that form the inner coat of the new virus. Then the viral polymerases make complementary copies of each of the gene segments to yield the double-stranded RNA viral genome. After genome synthesis is complete, two more protein coats are added, and newly made rotaviruses exit the infected cell, leaving it dead or dying. Because viral mRNA is produced while the virus still has two coats on, and because the double-stranded genomes of new viruses are constructed only after the virus has put on its first coat, rotavirus can be said to “replicate under cover.”

Viral Spread

Because of its three protective coats, rotavirus is able to thrive in the harsh environment of the digestive tract. And the requirement for intestinal enzymes to facilitate the “unbuttoning of the virus overcoat” helps explain why rotavirus doesn’t establish infections in other parts of the body—areas where these enzymes are lacking. The favorite targets for a rotavirus infection are the columnar epithelial cells at the tips of the villi that line the intestine. Because this is a cytopathic virus, a rotavirus infection leaves the infected villi looking like they have been chewed off. Why the rotavirus prefers the cells at the very tips of the villi isn’t clear. Perhaps these are the easiest cells for the virus to grab onto as it passes by, or maybe it has to do with the fact that the cells at the tips are the most “mature” epithelial cells in the villi. This maturity (whatever that means) may provide an environment within the cell that is especially well suited for rotavirus reproduction.

Although relatively few intestinal cells are attacked during a typical rotavirus infection, these infected cells crank out so much virus that the stool of an infected person can contain as many as one billion viruses per milliliter. In addition, rotavirus remains infectious suspended in water, so the virus can be spread in a contaminated water supply. Because as few as ten rotavirus particles can initiate an infection, it’s easy to understand how this virus spreads so efficiently by the fecal-oral route.

Rotavirus is especially contagious among young children. This makes sense: Young children produce feces almost continuously, and they like to put their mouths on everything in sight. Indeed, it is the rare four-year-old who has not been “visited” by this virus. Up until about three months of age, babies who have been breast fed are at least partially protected against a rotavirus infection by maternal antibodies. In this age group, infection is frequently asymptomatic, but some virus can still be produced and shed in the feces. Although maternal antibodies can protect against disease, these antibodies also can prevent babies from becoming immunized by the virus infection. This is because the virus is usually subdued by the inherited immunity before the child’s own immune system can mount a vigorous enough immune response to generate memory cells. Once this “passive” immunity from mom “wears off,” children become susceptible to infection. Consequently between the ages of six and forty-eight months, most children are infected with rotavirus.

Although rotavirus infections are observed year round, an interesting feature of rotavirus infections is that there is an annual rotavirus epidemic which spreads like a broad wave across North America—starting in Mexico and ending up in the northeastern United States. All sorts of theories have been advanced to explain this wave-like spread, but no single explanation has proved convincing. This raises an interesting point: If rotavirus infections occur mainly once a year, where is the virus hanging out during the “off season”? Rotaviruses can infect many different animals and birds, but no animals have been identified that can efficiently pass type A rotavirus to humans. So if there is an animal reservoir, it has yet to be discovered. It is more likely that rotavirus just “hibernates” in dried stool until conditions are right for it to spread.
again. Indeed, because of its triple coat, rotavirus is resistant to dehydration, a condition that is lethal to less-well protected viruses. So rotavirus is perfectly adapted for lying dormant until the next kid with an appetite for feces happens by.

**Evading Host Defenses**

Double-stranded RNA is a potent trigger for interferon production, so we might expect that infection with a double-stranded RNA virus like rotavirus would lead to the production of huge amounts of interferon. However, when rotavirus enters a cell, its double-stranded RNA genome is protected from "view" by its two inner coats. Transcription of viral mRNA takes place within these coats, and single-stranded viral RNA is squirted out into the cytoplasm. Then, when it is time to produce new double-stranded genomes, the single-stranded RNA is first enclosed in a coat of protein, and only then is the second strand of RNA produced. As a result, double-stranded viral RNA is not readily "visible" within the cell, and consequently, relatively little interferon is produced during a rotavirus infection. So rotavirus' "replication under cover" strategy helps it evade the host’s interferon defense, buying time for the virus to reproduce and spread.

Because the infected cells die as a result of a rotavirus infection, the innate system is quickly alerted to deal with the attack, and the adaptive system cranks up to produce protective antibodies. Although these antibodies may play an important role in "mopping up" any residual rotaviruses, by the time the immune system really gets going, most of the rotaviruses either have been killed by the innate system or have exited with the feces. Thus, rotavirus is a “hit and run” virus that replicates quickly, and produces a large number of new viruses which then are quickly swept away in the feces to infect the next victim.

Partly because the immune system only gets a “glimpse” of the rotavirus, and partly because the immune response generated by most mucosal invaders seems to be rather short lived, a rotavirus infection usually does not generate complete immunity to a subsequent infection. What this means is that older children and adults frequently can be re-infected with the same rotavirus strain they fought off as a kid. In many of these re-infections, there is enough immunological memory left to prevent most of the symptoms usually associated with a first infection. Even so, infectious virus can be shed in the feces of asymptomatic individuals, enhancing viral spread.

Like other RNA viruses, the rotavirus genome has a high rate of mutation, and some of these mutations can change the parts of the viral coat that are recognized by neutralizing antibodies. As a result of this antigenic drift, there are always several different rotavirus serotypes circulating in the population.

**Viral Pathogenesis**

In infants and young children, rotavirus is the major cause of severe inflammation of the intestine (gastroenteritis). Indeed, about one third of the cases of diarrhea severe enough to result in the hospitalization of young children are caused by rotavirus infections. Worldwide, this virus causes nearly a million deaths each year, mostly in underdeveloped countries.

Rotavirus infections frequently result in fever, vomiting, and diarrhea. Early on, it was believed that rotavirus-associated diarrhea was the direct result of the killing of villus intestinal cells by the virus. This killing was thought to cause an imbalance between the fluid-secreting cells in the crypts at the base of the intestinal villi and the mature absorptive cells at the tips of the villi. However, it was later learned that rotavirus usually infects a relatively small fraction of the villus cells in the digestive tract, so cell killing could not be the whole story. Next, it was hypothesized that a toxin encoded by the virus acts directly on intestinal cells (e.g. on crypt cells) to cause diarrhea—but experimental evidence for such a direct-acting viral toxin is lacking. Indeed, the latest experiments suggest that much of the fever, vomiting, and diarrhea that result from a rotavirus infection are caused indirectly through the action of the nervous system. Here’s how this is thought to work.

It has long been known that vomiting involves a “reflex loop” which begins when nerves that have their inputs in the walls of the gastrointestinal tract sense that the gut is inflamed. These inflammatory signals are then
transmitted by the nervous system to the “vomiting center” in the medulla. If these transmissions are of sufficient strength, neuronal signals are then sent out from the medulla, initiating a rather complex series of events that leads to vomiting. This includes a deep breath, followed by contraction of the diaphragm and the abdominal muscles, and opening of the esophageal sphincter to allow the vomit to escape. This vomit reflex, of course, is a host defense that is designed to help clear the upper gastrointestinal tract of invaders.

Recent experiments in mice now suggest that the diarrhea associated with a rotavirus infection also involves a neuronal reflex loop. In the intestines, there are many activities which must be carefully monitored and coordinated. For example, just the right quantities of digestive enzymes must be released into the small intestine from the pancreas, and just the right amounts of mucus and fluid must be provided by the cells that line the intestines. In addition, the peristaltic contractions of the muscles that line the intestines must be coordinated so that the contents of the intestines are propelled in the correct direction. Reverse peristalsis would not be good. All of these functions are controlled by what neuroscientists call the enteric nervous system. This system has sensors that provide up-to-date information on the environment in the intestines, a processing unit that can make decisions based on this information, and neural outputs that can implement these decisions. Most remarkably, all this can be accomplished without ever sending a signal to or receiving a signal from the brain.

The current thinking about rotavirus-induced diarrhea is that somehow the viral infection stimulates the receptors of the enteric nervous system that are located in the small intestine. If this “I’ve been attacked” signal becomes strong enough, the diarrhea center in the “gut brain” reacts by generating neural signals that stimulate the crypt cells at the base of the intestinal villi to secrete more fluid into the intestine, causing diarrhea—a defense mechanism meant to help clear invading pathogens from the gut.

So far it is not clear how a rotavirus infection triggers the attack signal. There is some evidence that a rotavirus protein (NSP4), which normally is involved in assembling its coat, binds to receptors on specialized “sensor” cells in the walls of the small intestine. These sensor cells may then react by secreting substances (e.g., serotonin or prostaglandins) that stimulate nearby nerve endings. Another possible (and less elaborate) explanation is that the inflammation caused by the killing of rotavirus-infected epithelial cells provides the trigger for the vomit and diarrhea reflexes.

Fever, the third common symptom of a rotavirus infection, was originally thought to be due to inflammatory cytokines such as interleukin-1 that travel from the site of infection to the brain, where they can trigger an increase in body temperature. Although this may be true in some cases, recent experiments suggest that inflammatory cytokines can also stimulate nerves in the intestine which then carry the “under virus attack” signal directly to the fever center of the brain. Indeed, the emerging picture is that most of the symptoms of a rotavirus infection—fever, vomiting, and diarrhea—likely result because viral proteins or virus-associated killing of intestinal cells triggers neuronal reflex loops.

It is important to remember that these symptoms are the unintended consequences of the lifestyle choices made by the rotavirus. Fever, vomiting, and diarrhea are host defense mechanisms, and activating these defenses is certainly of no value to the virus. Indeed, even during asymptomatic rotavirus infections, large amounts of virus can be produced and shed in the feces.

**ADENOVIRUS—A VIRUS WITH A TIME SCHEDULE**

The second enteric virus we will discuss is the human adenovirus. Actually, human adenoviruses comprise a large family of viruses with about fifty different serotypes. In addition, there are adenoviruses that infect many kinds of birds and animals. Even frogs get adenovirus infections. So far, however, there have been no reports of adenovirus transmission between humans and other species.
Although most human adenovirus serotypes cause infections of the respiratory tract, adenovirus serotypes 40 and 41 specialize in infecting cells that line the intestines. These “enteric adenoviruses” will be our focus here.

**Viral Reproduction**

The adenovirus genome is a linear, double-stranded, DNA molecule with enough genetic information to encode over thirty proteins—so we can expect this virus to have a number of “bells and whistles” that smaller viruses don’t have. In fact, these “extra genes” are what make adenovirus so interesting. In contrast to rotavirus, which has three protein coats, adenovirus only has one. Sticking out of this relatively smooth capsid are “fiber” proteins that give the adenovirus the appearance of a communications satellite.

As you might predict, the knobs at the end of the fiber stalks plug into cellular receptor molecules and allow the virus to attach to its target cell. This attachment, however, is not enough to permit the virus particle to enter the cell. In fact, the proteins that make up the base of the fiber stalk, the “penton base,” must also plug into co-receptor molecules on the cell surface. Only when this second contact is made can the virus be taken into the cell, enveloped by an endosome.

After the virus is partially dismantled by the acidic conditions within the endosome, it escapes into the cytoplasm, and injects its cargo of DNA into the cell’s nucleus. You may be wondering: If the virus coat is “partially dismantled” in the acidified endosome, why isn’t the virus stripped totally naked by the acidic conditions in the stomach? I could wave my hands and make up a story about how the virus may be protected within a clump of food as it transits the stomach, but the truth is, nobody has a clue as to how adenovirus accomplishes this amazing feat.

Anyway, once the viral DNA reaches the nucleus, cellular enzymes begin to transcribe some of the viral genes into mRNA. Transcription of adenoviral genes proceeds according to a carefully orchestrated program, with certain viral genes being transcribed early after infection, and others being transcribed at later times. It is this ability of adenovirus to function on a strict time schedule that makes it such an effective parasite.
Because both the adenoviral genome and the cell’s DNA are linear and double-stranded, the simplest strategy for adenoviral DNA replication would be to just use the cell’s DNA replication machinery. After all, why reinvent the wheel? But no. Adenovirus is not content with this “simple” approach. The fact is, no virus exists which uses exactly the same strategy for DNA replication as human cells do—it just won’t work for viruses. One major problem has to do with the timing of DNA replication.

When cells proliferate, cellular DNA replication is carefully controlled so that each origin of replication is used only once during a cell division cycle. This insures that each chromosome is copied only once, and that each of the two daughter cells receives one complete copy of the genetic information, and no more. This scheme works just fine for cells, but if viruses used this strategy, only one new virus could be produced per cell cycle. Since viruses make their living by using the cells they infect to make thousands of copies of their genomes, one copy per cell division just won’t cut it.

So DNA viruses like adenovirus must somehow uncouple their replication cycle from that of the cells they infect. Adenovirus accomplishes this by using its own DNA polymerase to replicate in a way that is totally different from that of cellular DNA. When cells replicate their DNA, a cellular DNA polymerase moves along one parental strand constructing a continuous complementary daughter strand. At the same time, a DNA polymerase copies the other parental strand. However, because the DNA polymerase only works in one direction, copying this second parental strand results in small pieces of DNA which must subsequently be joined together. So replication of cellular DNA is continuous on one strand and discontinuous on the other.

In contrast, during adenoviral DNA replication, the virus’ polymerase makes a complementary copy of one parental strand, displacing the second parental strand. The result is one double-stranded viral DNA molecule plus the displaced single strand. This displaced parental strand is then copied by the viral DNA polymerase to make a second double-stranded molecule. With this scheme, the replication of both strands of viral DNA is continuous.

Another difference between cellular and adenoviral DNA replication is that the cellular DNA polymerase requires a short piece of RNA to “prime” DNA synthesis. In contrast, adenoviral DNA replication is primed by a viral protein that binds to one strand at each end of the viral DNA. As a result, replication of adenoviral DNA doesn’t have to wait for an RNA primer to be synthesized. And because adenoviral DNA is symmetrical as far as the protein primers are concerned, DNA replication can actually begin at either end. In fact, if there are plenty of replication goodies available, two polymerase molecules can roar along the viral DNA in opposite directions, simultaneously copying both strands.
The whole process of adenoviral DNA replication employs not only several viral proteins (e.g., the viral DNA polymerase and primer proteins), but cellular proteins as well. In fact, to replicate its DNA, adenovirus uses many of the supplies that the cell normally would use for replication of its own DNA. These include proteins involved in the copying operation as well as the nucleotide building blocks that are hitched together to form new DNA molecules. This dependence on cellular replication factors raises a potential problem: Many cells the virus would like to infect are not proliferating, and when cells are in a “resting” state, they generally don’t keep large quantities of the materials needed for constructing DNA molecules on hand.

To solve the “resting target cell” problem, adenovirus produces, immediately after infection, proteins (the E1A proteins) that kick the infected cell into its DNA replication cycle. When this happens, the cell begins to stockpile the materials the virus will need to make many copies of its DNA. Of course, while these DNA replication supplies are being amassed, it wouldn’t do to have the cell replicate its DNA and use them all up. No indeed. So to keep this from happening, the virus shuts down synthesis of cellular DNA. Adenovirus can play this dirty trick because the mechanisms for adenoviral DNA replication and host cell DNA replication are very different.

So adenovirus employs a novel DNA replication strategy which uncouples viral DNA replication from the host cell’s replication cycle. This makes it possible for many cycles of viral DNA replication to take place in the time it would normally take the cell to replicate its DNA only once. In addition, the novel replication strategy allows the virus to trick the host cell into making lots of supplies for DNA replication—and then to use them all for viral replication.

After the raw materials required for making DNA have accumulated, the virus begins to make mRNAs that encode its DNA polymerase and the other proteins required for viral DNA replication. This delay makes sense. There’s no reason for the viral DNA polymerase to begin replication until the materials needed for the job are available. Finally, about eight hours after the virus first enters the cell, synthesis of new viral DNA begins within the cell nucleus.

Usurping the cellular DNA replication machinery is not the only “takeover” strategy used by adenovirus. Soon after infection, the virus makes two proteins (the E1B-55K and E4 ORF6 proteins) which interfere with the transport of cellular, but not viral, mRNAs out of the nucleus. This selective transport of viral RNA insures that viral proteins with enzymatic functions (e.g., the polymerase) are available early on. Then later, when a huge number of proteins must be produced to build thousands of viral capsids, the virus uses another tactic: It rigs things so that translation of viral mRNAs is heavily favored over translation of cellular mRNAs. Focusing the cell’s machinery on viral protein production late in infection is important, because construction of each capsid requires over 1,600 protein molecules!

The mechanisms involved in the selective transport and translation of viral mRNAs are not well understood. However, the net result of this takeover is that during the final hours of an adenovirus infection, over 90% of the protein synthesized is viral. So adenovirus not only takes over DNA synthesis in infected cells, it also commandeers the cell’s protein synthesis machinery. All this trickery relies on carefully timed expression of viral proteins, and makes adenovirus replication so efficient that a single infected cell can make roughly 100,000 virus particles. This is ten to one hundred times as many virus particles as are produced by cells infected with most other viruses.

**Virus Spread**

Like rotavirus, enteric adenoviruses spread by the fecal-oral route, and young children are the main targets of enteric adenovirus infections. Interestingly, although many human cells have receptors for adenovirus on their surfaces, enteric adenovirus infections do not spread beyond the digestive tract. This is certainly a good thing. If adenovirus, which produces huge amounts of new viruses during an infection, were to spread throughout the body of a young child, the result would most likely be devastating—both for the child and the virus. After all, a virus that spreads efficiently by the fecal-oral route and kills all the young children it infects would not be a very successful human pathogen.

Although there probably are several factors that contribute to the fortunate containment of enteric adenoviruses within the gastrointestinal tract, one important ingredient is the lack of adenovirus receptors on dendritic cells. If adenovirus could infect dendritic cells, be transported by the infected cells to nearby lymph nodes, and crank out hundreds of thousands of new virus particles, a life-threatening systemic infection would probably result.

**Evading Host Defenses**

Although each adenovirus-infected cell produces a huge number of new viruses, the process is relatively slow, taking about two days from the time the virus enters until
most newly made viruses exit the infected cell. This contrasts dramatically with the rotavirus, which takes only about six hours to turn out a new crop of viruses. This rather leisurely pace of adenovirus reproduction makes adenovirus-infected cells vulnerable to attack by host defenses. Consequently, adenovirus must take effective countermeasures to ensure that viral reproduction can be completed before infected cells are destroyed. In fact, roughly one quarter of the adenovirus genome is dedicated to countering host defenses.

All cells have “alarm systems” that alert them when biosynthesis is not going as planned, and which trigger a cascade of events within the cell that leads to the cell’s death by apoptosis. This type of quality control is necessary, because a cell’s biochemical systems are so complicated that dysregulated cells are a frequent occurrence—and these out-of-control cells can pose a real threat to the human organism (e.g., by leading to cancer). Because adenovirus totally usurps the biosynthetic machinery of the cells it infects, you can be certain that these alarms go off. And if the virus did nothing to prevent it, apoptosis programs would surely be set in motion that would kill the cell and the virus within it long before the virus could reproduce.

At least two adenoviral proteins are devoted to dealing with the host’s “suicide” defense against stressed cells. As is appropriate, both are expressed early after infection. One (the E1B-55K protein) blocks transcription of cellular genes that normally would activate the death program. The other (E1B-19K) binds to and inactivates key host proteins involved in initiating apoptosis. These apoptosis inhibitors make it possible for the virus to disrupt normal cell activities without the cell responding to the “something is terribly wrong” alarm.

Suppression of apoptosis during an adenovirus infection is an excellent idea, because a dead cell isn’t going to produce much virus. But this suppression also poses a potential problem. Although an adenovirus-infected cell is pretty beat up by the time viral reproduction is complete, unless something is done to really rip the cell open, the newly made viruses will just slowly dribble out. Viruses like rotavirus, which reproduce quickly, actually use apoptosis to help accomplish their “final exit” from the cell. But adenovirus, because it suppresses apoptosis, must make other exit arrangements.

Late in infection, when virus assembly is nearly finished, adenovirus produces a protein aptly named “the adenovirus death protein” (E3-11.6K). This protein acts in a still-mysterious way to burst open the infected cell and allow the 100,000 new viruses trapped inside to come roaring out. The importance of this very late-appearing protein is demonstrated by the discovery that adenovirus mutants which cannot make the death protein take about an extra week to exit an infected cell. So the E1B proteins, which are made early in infection, hold off cell death long enough for viral reproduction to be completed. Then, just when newly made viruses are ready to exit, the adenovirus death protein delivers the coup de grace. Yes, timing is everything.

Another peril that adenovirus must face is the interferon system. It might seem that this virus would not induce interferon production in the cells it infects. After all, adenovirus has no lipid envelope, and its genome does not replicate through a double-stranded RNA intermediate. However, adenoviral mRNAs are transcribed from both strands of the viral DNA genome, and to take full advantage of its coding capacity, the virus allows some of these coding regions to overlap. That way the same stretch of DNA can produce two different mRNAs—one from one strand and a second mRNA from the other, complementary strand. One result of this arrangement of genes, however, is that mRNAs transcribed from these overlap regions have complementary sequences which can base pair to produce long, double-stranded RNA molecules. So by positioning genes opposite each other on its two DNA strands, adenovirus increases its capacity to make proteins. But it does so at a cost—exposure to the interferon defense system.

To protect itself from the interferon it induces, adenovirus produces “decoy” RNA molecules called VA RNA. Normally, in interferon-alerted cells, double-stranded viral RNA binds to the sensor protein, PKR. It is this binding which activates PKR (a protein kinase), and shuts down protein synthesis, ending the viral infection. However, in adenovirus-infected cells, the VA RNA binds to PKR and renders the protein kinase inactive, allowing protein synthesis to continue.

In addition to producing molecules that help the virus cheat death at the hands of the apoptotic and interferon “executioners” within the cell, adenovirus must also defend itself against attacks from outside the infected cell. Indeed, because adenovirus reproduces slowly, adenovirus-infected cells should be attractive targets for destruction by killer T cells. Killer T cells recognize fragments of viral proteins displayed by class I MHC molecules on the surface of an infected cell. Because adenovirus makes so many different proteins in such abundance, there is no way that fragments of some of these proteins won’t fit nicely in the grasp of class I MHC molecules and be put on display on the cell surface. Such an advertisement of the infected status of the cell would likely result in its destruction by killer T cells, well before new viruses could be produced.
As a defense against killer T cells, adenovirus has evolved a mechanism that keeps viral proteins from being displayed on the surface of virus-infected cells. Normally, class I MHC molecules are loaded with protein fragments in the endoplasmic reticulum from whence they proceed to the cell surface to display their cargo. However, in adenovirus–infected cells, a viral protein (E3-19K), which is anchored firmly in the endoplasmic reticulum, grabs class I MHC molecules and prevents them from traveling to the cell surface. This “not so fast, Buster” strategy works nicely, because if killer T cells don’t see viral proteins displayed on the cell surface, they have no way of knowing that the cell has been infected. Of course, the E3-19K proteins can’t snag every single class I MHC molecule as it passes by, so this evasion strategy isn’t perfect. But it does greatly decrease the destruction of adenovirus-infected cells (and the viruses inside them) by killer T cells.

But wait! Won’t preventing the expression of class I MHC molecules on the cell surface make adenovirus-infected cells excellent targets for attack by natural killer cells? After all, natural killer cells do specialize in destroying cells that don’t have class I MHC molecules on their surfaces. Fortunately, the clever adenovirus has evolved ways to deal with at least one of the weapons carried by natural killer cells.

Killer T cells and natural killer cells both have two different ways to kill cells. First, these killers can trigger death by apoptosis when proteins on their surfaces (e.g., FasL) plug into “death receptors” (e.g., Fas) on the surfaces of their targets. To counter this weapon, adenoviruses make a protein called RID that binds to the death receptors on adenovirus-infected cells, removes them from the cell surface, and oversees their destruction. As backup, the adenovirus makes another protein (E3-14.7K) that interrupts the signal from the death receptors—just in case RID misses a few. As a result of the action of these two virus proteins, one of the potent weapons used by both killer T cells and natural killer cells is effectively neutralized.

The second way killer T cells and natural killer cells destroy virus-infected cells involves enzymes called granzymes that these killers “squirt” into their target cells. So far, nobody has discovered a strategy that protects adenovirus-infected cells against this kind of killing. In fact, in all the world of viruses, there is not a single example of a virus that has evolved a defense against granzyme-mediated killing. From the virus’ perspective, this is probably a good thing. If the adenovirus were completely protected against killing by killer T cells and natural killer cells, its human hosts would be almost defenseless against the viral infection. Because a dead host is usually not a good host, humans and adenoviruses probably have reached a standoff in which adenovirus has evolved just enough countermeasures to allow it to reproduce efficiently without seriously damaging its hosts.

Human adenoviruses have about fifty different serotypes, defined by neutralizing antibodies which recognize different versions of two of the proteins that make up the viral capsid (fiber and hexon). So it would seem that the adenovirus DNA polymerase must be error-prone. Indeed, although experiments indicate that the viral DNA polymerase has some capacity to “proofread” its work, the adenovirus polymerase certainly isn’t nearly as error-free as the cellular DNA polymerase—an enzyme complex that makes only about one mistake per 100 million bases. So adenovirus had another good reason for not using the cellular DNA polymerase to replicate its genome: By using its own polymerase, adenovirus is able to generate antigenic drift, albeit probably not as rapidly as RNA viruses like rotavirus.

Pathogenic Consequences of an Enteric Adenovirus Infection

Enteric adenovirus infections are second only to rotavirus infections as the most frequent cause of infantile diarrhea, and by the age of three, most children have been infected by an enteric adenovirus. Although rotaviruses and adenoviruses are extremely different in terms of their reproductive strategies and evasion tactics, both viruses spread by the same route, infect and kill the same cells, and do not cause disseminated infection. As a result, in the clinic, it is usually impossible to differentiate between an enteric adenovirus infection and a rotavirus infection based on symptoms alone.

Although the symptoms of adenovirus and rotavirus infections are the same—fever, vomiting, and diarrhea—the time courses of these two infections are different. The reason, of course, is that the rotavirus reproduces very quickly, whereas the adenovirus takes its sweet time. Usually about a week elapses between an adenovirus infection and the appearance of any symptoms. This makes sense, because it takes several days for even the first adenovirus-infected cells to begin to produce virus. In contrast, a week post infection, the rotavirus has “left the building,” and the symptoms of a rotavirus infection have usually abated. Not only do the symptoms of an enteric adenovirus infection appear later, these symptoms generally last longer than those of a rotavirus infection. This is because, in contrast to the
“hit and run” rotavirus, the adenovirus has invested heavily in tactics that allow the virus to evade host defenses for a relatively long time. Eventually, the host’s immune system does deal harshly with an enteric adenovirus infection, and the virus is banished (cleared) from the host. And because the adenovirus doesn’t “go gentle into that good night,” the adaptive immune system becomes fully activated, and immunity to the infecting strain of adenovirus is long-lasting. This is quite different from the situation with a rotavirus infection in which the adaptive immune system only gets a glimpse of the virus, and as a result, immunity to subsequent infections is usually incomplete.

In this lecture we have concentrated on two adenovirus serotypes, 40 and 41, that cause gastrointestinal disease. However, adenovirus got its name because it was first isolated from human adenoid tissue, and many adenovirus serotypes do cause upper respiratory infections. Still others cause childhood pneumonia, and some, as we have discussed, cause gastroenteritis. Interestingly, some adenovirus strains, for example serotypes 4 and 7, can infect both the respiratory tract and the gastrointestinal tract. Immunologists take advantage of the “dual targets” of these two serotypes when they vaccinate army recruits to prevent respiratory infections. That vaccine is made by packaging live adenovirus 4 and 7 in gelatin capsules, which are then swallowed by recruits. Administered in this way, the viruses in the vaccine bypass the respiratory tract, where they would cause acute respiratory disease, and go on to establish an asymptomatic, immunizing infection of the epithelial cells of the small intestine. What’s so elegant about this vaccination strategy is that the immunity generated by this asymptomatic intestinal infection protects not only against future intestinal infections, but also against respiratory infections by serotypes 4 and 7.

**HEPATITIS A—A VIRUS THAT DETOURS**

According to most estimates, over half the population of the United States has been infected with hepatitis A virus, so we certainly need to include this one in our Parade. But what really makes this virus so interesting is that, although it enters its host through the mouth and exits through the anus, just like rotavirus and the enteric adenoviruses, on its trip from top to bottom it takes a “detour” through the liver. It is this detour which makes hepatitis A virus such a successful human pathogen. It is also the main feature of the virus lifestyle that leads to the pathological consequences of a hepatitis A virus infection.

**Viral Reproduction**

Hepatitis A virus consists of a single piece of positive-strand RNA encased in a single capsid made of protein—just like the rhinovirus. In fact, these two viruses are so similar in their organization that it is widely assumed they reproduce in very similar fashions. However, there is at least one major difference in the ways rhino and hepatitis A viruses reproduce. Whereas rhinovirus shuts down synthesis of host proteins, killing the cells it infects, hepatitis A virus reproduces “gently,” making new viruses without causing perceptible damage to its host cells.

**Viral Spread**

Another important difference between rhinovirus and hepatitis A virus is that the capsid of hepatitis A virus is resistant to the acid conditions in the stomach—conditions that would destroy the rhinovirus capsid. As a result, rhinovirus is a respiratory virus, and hepatitis A virus usually is spread by the fecal-oral route. This illustrates the important concept that subtle changes in viral design can result in major differences in the route of entry of a virus and in the diseases that result from a virus infection.

Hepatitis A is a virus that targets the liver—an organ through which blood flows continuously. So we might predict that this virus would be spread when humans exchange blood or blood products (e.g., during a blood transfusion or when drug abusers share needles). However, hepatitis A virus usually is eliminated quickly by a patient’s immune system, and hepatitis A virus never establishes a chronic infection. As a result, the period of time when an infected individual’s blood contains hepatitis A virus is generally so short that the probability of spread by blood to blood contact is quite small.

In contrast, hepatitis A virus is well suited to be spread efficiently by the fecal-oral route. After four weeks in dried feces, its infectivity only decreases by about a factor of 100. In addition, the virus can survive for weeks in shellfish, which can concentrate the virus by filtering large volumes of contaminated water. Fortunately for us, hepatitis A virus is sensitive to the concentrations of chlorine commonly used for water treatment (and also to toilet bowl cleaner!).

There is only one known serotype of hepatitis A virus, and infection generally leads to life-long immunity. So hepatitis A virus depends on lax hygiene and a large population of susceptible individuals for its continued existence. Although several types of animals can be infected with hepatitis A experimentally, there is no known natural animal reservoir for this virus.
Evading Host Defenses

As you would expect for a virus which is primarily spread by the fecal-oral route, hepatitis A enters cells in the small intestine. However, infection of these cells has been extremely difficult to document. Moreover, newly made virus is usually not detected in feces until weeks after a hepatitis A infection. This suggests that very few intestinal cells are infected, and that they produce relatively few new virus particles. Now of course, if this were the complete story, hepatitis A virus would be in deep trouble in terms of persisting in the human population. However, this cunning virus has a trick up its sleeve that makes it all work nicely: Hepatitis A virus takes a “detour” to the liver on its way through the digestive tract, and in doing so, evades host defenses long enough to become one of the world’s leading pathogenic viruses.

It has been known for years that the primary target of a hepatitis A virus infection is the liver. That, of course, is why it’s called a hepatitis virus (hepato is Greek for liver). In contrast to its infection of intestinal cells, which is pretty wimpy, a hepatitis A infection of liver cells is robust, producing large quantities of virus. These newly made viruses are released into the bile ducts that drain the liver, and are subsequently emptied into the intestines with the bile. In fact, production of hepatitis A virus by liver cells is so efficient that at the height of a hepatitis A infection there can be 100 million virus particles excreted per milliliter of feces. That’s a lot of virus! Although it is clear that most hepatitis A virus infections begin in the intestine, that the virus then detours through the liver, and that newly made viruses are delivered back to the intestine with the bile, the big question has been, “How do the small number of virus particles generated during an intestinal infection ever manage to make their way to the liver and establish an infection there?” Very recent experiments have suggested an elegant solution to this longstanding problem. Here’s how it is believed to work.

Keeping watch over our intestines is the mucosal immune system. Although the “rules” that govern this arm of the immune response are not as well understood as those which apply to the immune system in other parts of the body, a picture of how mucosal immunity works is starting to come into focus. It is likely that when hepatitis A virus infects cells that line the intestines, some of the newly made viruses are transported through specialized M cells into the tissues below. These M cells are tasked with sampling the contents of the intestines, and with helping to initiate an immune response to invaders. From the tissues beneath the M cells, the virus is transported to nearby lymph nodes where B cells are activated which can produce antibodies that can bind to the virus. This process takes a week or more while the selected B cells proliferate to build up their numbers, travel back to the tissues underlying the intestines, and begin to pump out antibodies specific for hepatitis A virus. These antibodies are generally of the IgA class, because this class of antibody is especially well suited to defend against intestinal invaders. IgA antibodies can be transported into the intestine itself, bind to newly made viruses, and shepherd them out of the intestine with the feces. And IgA antibodies can also bind to viruses which have invaded the tissues that surround the intestines. However, IgA antibodies have one important weakness—a weakness that hepatitis A virus has learned to use to its advantage.

Other antibody classes (e.g., IgG antibodies) can form a bridge between an invader and a professional phagocyte (e.g., a macrophage), making it easier for the phagocyte to “eat” the invader. However, IgA antibodies perform this maneuver with great difficulty, because phagocytic cells have only low-affinity receptors on their surfaces for IgA antibodies. As a result, IgA antibody-virus complexes are not efficiently “cleared” from the tissues by phagocytic ingestion. Rather, IgA antibody-virus complexes in the tissues are collected by the lymphatic system, poured into the bloodstream, and sent to the liver. Liver cells (hepatocytes) do have receptors for IgA antibodies (asialoglycoprotein receptors), and once connected to these receptors, both the IgA antibodies and their cargo of invaders are taken inside the liver cell for disposal.

It now appears that hepatitis A virus actually uses this IgA “disposal system” to solve its problem of how to infect liver cells. As IgA antibody–virus complexes collected from intestinal tissues travel to the liver for disposal, hepatitis A virus happily goes along for the ride. And when the IgA antibody–virus complexes reach the liver, the virus uses the uptake of these complexes to usher it into the very cells it wants to infect. Once inside hepatocytes, the virus somehow escapes destruction, and begins to reproduce. Newly made viruses are then released by the infected liver cells and sent back into the intestines along with the bile that the liver generates. This return trip to the intestines is a piece of cake for this virus, because the protein coat of hepatitis A is unfazed by the bile salts, which act as detergents and would destroy “ordinary” viruses. But hepatitis A is no ordinary virus!

It gets even better, however. Like politics, immune responses are usually local. They have to be, because the human body is continually under attack on
Viral Pathogenesis

Because the initial infection of cells in the small intestine is so gentle, the early phase of a hepatitis A infection is uniformly asymptomatic. A week or two into the infection, the virus makes its way to the liver, and viral particles produced by infected liver cells begin to be excreted in the feces. After another week or so, killer T cells, activated in response to the liver infection, start to appear, and the destruction of infected liver cells begins. Because hepatitis A is not a cytolytic virus, this cell killing results from the immune response to the infection, not from the viral infection itself. Fortunately, the number of hepatitis-A-infected liver cells is usually too small to compromise liver function. Consequently, most hepatitis A infections, especially in young children, are asymptomatic.

In a minority of hepatitis A infections, mainly in adults, the destruction of liver cells is more extensive, and symptoms characteristic of viral hepatitis begin to appear about four weeks post infection. Certainly the most striking hepatitis symptoms are jaundice and dark urine. When your eyes turn yellow and your urine runs dark, you know something ain’t right.

In the human body, about 100 billion aged red blood cells are “retired” each day. These effete cells are rapidly digested by macrophages, and the iron they contain is recycled. However, the part of the hemoglobin molecule that cradles the iron atom cannot be reused, and after it has been processed by the macrophage to form a yellow pigment called bilirubin, it is spit out into the blood or tissues surrounding the macrophage. Because each red blood cell...
contains so many hemoglobin molecules, and because so many red blood cells are destroyed each day, the huge amount of bilirubin produced creates a major disposal problem. To deal with this, most of the bilirubin is complexed with proteins in the blood (primarily albumin) to make it soluble, and is carried to the liver. There the bilirubin is taken up by hepatocytes, modified, and released into the bile. When the system is working properly, disposal by the liver is so efficient that the concentration of bilirubin in fluids and tissues remains low. However, when large numbers of liver cells are destroyed by the immune response to a hepatitis A infection, this disposal system can be overwhelmed. When that happens, bilirubin concentrations increase dramatically, resulting in jaundice and dark urine.

Although bilirubin is not terribly toxic, jaundice and dark urine are pretty good indicators that the liver is not functioning properly. Because the liver is tasked with detoxifying many other waste products of normal cellular metabolism, jaundice is usually accompanied by symptoms such as malaise, loss of appetite, fever, nausea, and vomiting—symptoms which are caused by inadequate detoxification of cellular waste products due to liver damage. Fortunately, hepatitis A virus never establishes a chronic infection, the immune system usually requires only a few weeks to eradicate the virus, and liver cells destroyed by the immune response are quickly replaced by the proliferation of healthy liver cells. As a result, symptoms associated with a hepatitis A infection are generally short lived, and this virus rarely causes life-threatening disease. In the United States, for example, there are only about 100 deaths each year associated with hepatitis A infections, and these are mainly in older age groups.

So the detour that hepatitis A virus takes on its way from mouth to anus makes it possible for the virus to cause an acute infection of the liver. This infection is easily dealt with by the immune system before much liver damage has occurred, but after a large number of new viruses have been pumped out with the feces—viruses which can then go on to infect other humans via the fecal-oral route.

Table 4.1 reviews how our three model enteric viruses solve their problems of reproduction, spread, and evasion.

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<thead>
<tr>
<th>Reproduce</th>
<th>Spread</th>
<th>Evasion</th>
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<tbody>
<tr>
<td>Rotavirus</td>
<td>Enteric Adenovirus</td>
<td>Hepatitis A</td>
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<tr>
<td>Segmented, double-stranded RNA genome</td>
<td>Large, linear, double-stranded DNA genome</td>
<td>Positive, single-stranded RNA genome</td>
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<tr>
<td>Has three protein coats</td>
<td>Single protein capsid</td>
<td>Single protein capsid</td>
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<tr>
<td>Replicates “under cover” within its coats</td>
<td>Replicates slowly, using carefully timed plan of viral gene expression</td>
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<tr>
<td>Uses digestive enzymes to prepare virus for entry</td>
<td>Takes over control of DNA and protein synthesis in infected cells</td>
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<tr>
<td>Cytolytic</td>
<td>Cytolytic</td>
<td>Non-cytolytic</td>
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<tr>
<td>Infects cells at tips of intestinal villi</td>
<td>Infects intestinal epithelial cells</td>
<td>Initially infects intestinal cells; then infects liver cells</td>
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<tr>
<td>Fecal-oral spread</td>
<td>Fecal-oral spread</td>
<td>Enters digestive tract; “detours” through the liver; exits with feces</td>
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<td>Causes acute infection</td>
<td>Causes acute infection</td>
<td>Causes acute infection</td>
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<td>Hides from interferon system by replicating under cover</td>
<td>Viral proteins hold off apoptosis until replication is complete</td>
<td>Confuses immune system by detouring through liver</td>
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<tr>
<td>Speedy replication—hit and run; immunity not complete</td>
<td>Virus produces decoy RNA molecules to trick interferon system</td>
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<td>Antigenic drift produces multiple strains</td>
<td>Interferes with viral antigen presentation by MHC molecules</td>
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<td></td>
<td>Neutralizes one of natural killer cell weapons</td>
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