Using Viral Genes to Fight Disease

Giving "old" viruses new "opportunities" may lead to biotechnology therapeutics

Stephen M. Edgington

We often think of a virus's ability to mutate rapidly as the means by which "new" viruses arise, evade the immune system, and turn into pandemic killers. While this may be true of a virus's ability to sustain its pathogenicity once it has infected a new host, according to Stephen Morse (Rockefeller University, New York), most often the virus that launches the "new" epidemic is not new at all. Rather, it is an "old" virus given a new opportunity.[1]

FIGURE 1. Virus with an attitude: The Mengo virus may prove useful for vaccine development and gene therapy. Image by Jean-Yves Sgro.

Morse's reasoning is simple: "From an evolutionary point of view, a virus needs to replicate stably within its host population and cause relatively little disease in the host if it is to sustain itself," he says. "One strategy viruses employ is to maintain a low virulence and do very little damage to their natural host." Morse cites as an example the Hantavirus epidemic in the Southwestern U.S. "Apparently, Hantavirus doesn't do a lot in its natural rodent host," he says. It is not until rodents carrying the virus come into regular contact with humans that the virus's immune-evading tactics prove lethal. "Once humans come in contact with the virus it can be deadly," he says. Ironically, most often the damage these viruses wreak in the new host results from the host's own inappropriate immune response.

Recent research suggests that drugmakers may learn to turn the table on these "new" epidemics by giving "old" viruses—or at least some of their genes—new therapeutic opportunities. By teasing out the genes that viruses use to evade a host's immune system, researchers are developing new vectors for vaccine development and gene therapy. This strategy has also demonstrated potential as a means to suppress transplant rejection—suggesting these "old" genes may also provide new switches for modulating immune-system response.

A Virus with an Attitude

Ann Palmenberg (University of Wisconsin, Madison) never set out to develop a new vaccine vector. She was simply curious about why the Mengo virus (see Figure 1), a cardiovirus normally found in mice, would carry a gene that seemed to announce to the cell it infected, "You are under attack." This billboard-size message was spelled out in a 400 ribonucleotide-long sequence of cytidines (Cs)—an unusually long poly-C tract. Since much shorter poly-C stretches of this type are known to mobilize the cell's immune response to viral invaders, Palmenberg was puzzled as to why the virus would be trying to draw attention to itself.

What seemed especially wrong with this picture was that the Mengo virus, a member of the larger picornavirus family, is known for its ability to maim and kill. "Mengo, and the picornaviruses in general, can be thought of as a family of viruses with an attitude," says Palmenberg. "It works like a small stealth bomber, moving in on its first target, the macrophages, and replicating with great speed before the immune system has detected it." These infected macrophages then travel throughout the host depositing virus in the brain, spleen, heart, and other vital organs. While death is the most common outcome, diabetes and still-born fetuses are typical outcomes in survivors.

According to Palmenberg, Mengo's "attitude" is further demonstrated by its ability to infect "anything with hair." While all mammals, including humans, are potential targets, Mengo's favorite hosts are rodents. This indirectly contributes to the virus's ability to sustain itself: Because rodents are an abundant food source in the mammalian world, the virus is most commonly transmitted when an infected rodent is eaten. Palmenberg jokes that the only thing that keeps Mengo from being a health problem in humans, "is our fondness for cooking mice before eating them."

Curious about what practical advantage the poly-Cs might deliver, Palmenberg decided to see if she could use molecular biology to tease apart this puzzle. She initially planned to construct a recombinant Mengo and then use it to test the poly-C region's function. This seemingly simple proposition turned into a three-year-long technical nightmare: Making an infectious cDNA and cloning it into plasmids proved difficult because the poly-C area was toxic to bacteria. Creating cDNA copies of the poly-C tracts lead to a spontaneous deletion of the poly-Cs when reverse transcriptase converted the natural RNA sequence to DNA.

Frustrated by these attempts to reconstruct the entire

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genome, Palmenberg, on a whim, decided to practice transfections with the poly-C deficient virus. When she inoculated several mice with this poly-C-lacking virus, she was surprised to find that the recombinants were not only functional, but also exhibited a very different level of infectivity from their full-length brethren. "While wild type virus normally killed the mice within three days," says Palmenberg, "we found that the recombinant Mengo, containing less than 30 Cs, not only attenuated the virus's ability to replicate, but also produced seroconversion in the host." Further testing demonstrated an even more unanticipated result: Once a mouse had been inoculated with this shortened recombinant, it seemed to have lifelong protection from lethal doses of the wild type Mengo.[2]

At first, Palmenberg thought there must have been some mistake. But subsequent experiments demonstrated that the results of this fortuitous "accident" were real: She was able to demonstrate that each subsequent deletion of 3 bases—below a 30-base threshold of poly-Cs—decreased pathogenicity by an order of magnitude. What's more, the attenuation was stable: She could harvest the virus from an inoculated mouse, passage it in tissue culture, and then inoculate a new mouse, and obtain the same result.

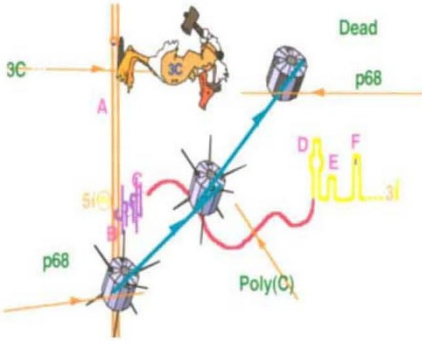

FIGURE 2. Poly-C tracts in the Mengo virus may serve as the "cheese" to trap protein kinases such as p68. This effectively knocks out the cell's first line of antiviral defense (see text).

A Better Mousetrap

But the question still remained: What is the function of the poly C tract? While the jury is still out, Palmenberg thinks she has a convincing argument about what may be taking place: She calls it the "poly-C mousetrap hypothesis." The assumption is that the poly-C tract serves as bait to trap the cell's first line of defense against viral attack—constitutively expressed cellular protein kinases (see Figure 2). These kinases serve as sentinels—roaming the cell in search of viral genes. If they discover what they think is a viral genome, they bind to it and become activated through a process of autophosphorylation. From there, their kinase potential is activated and they move about the cell setting off a series of phosphorylation-induced events: shutting down the cell's translation machinery to halt viral replication, initiating apoptotic pathways to destroy the infected cell, and inducing the virus-fighting abilities of interferon.

Palmenberg thinks that Mengo—along with the rest of the cardioviruses—may have hit on the strategy of developing long poly-C tracts as a way to short-circuit these events. Once the kinases attach themselves to this poly-C cheese, they become entangled in an RNA net that prevents the speedy commission of their cellular duties. "The idea is much like Paul Revere being caught and unable to get out of Boston to warn, 'the British are coming,'" says Palmenberg. The trap swings closed when either a viral protease or a cellular protease with housekeeping functions discovers the incapacitated kinase and cleaves it.

Shortening the length of the poly-Cs seems to boobytrap the virus's chances for survival in the wild. The virus starts to replicate, but as it does so, the protein kinases are allowed to do their job: As translation grinds to a halt, apoptosis kicks in, and interferon arouses B- and T-cell activity. What's more, the fact that this abortive infection is initiated in the macrophage population—the best cells for antigen presentation—assures the speedy distribution of the virus's description, in the form of peptide fragments, throughout the host. In the face of this coordinated attack, Mengo is eliminated within a week.

While the details of the poly-C mechanism are being worked out, Palmenberg is pushing ahead to develop recombinant Mengo as a universal vector for vaccines. So far, her progress is impressive. After demonstrating the effectiveness of the recombinant Mengo virus in mice, she went on to show that it could also protect domestic pigs, baboons, and macaques from the serologically related cardiovirus, encephalomyocarditis virus (EMCV). Again, these studies showed that single inoculations could produce lifelong immune protection.[3]

With her collaborators at the Institute Pasteur (Paris, France), she has also moved on to demonstrate that recombinant Mengo could prove to be effective in human disease. In a novel set of experiments, an RNA coding for 147 amino acids of the HIV-1's glycoprotein 120 (gp120) region was fused to the genome coding for the leader peptide of the Mengo recombinant. Since this nonstructural leader protein is cleaved by a viral protease, fusing the gp120 polypeptide forces the recombinant virus to introduce a foreign antigen into the infected cell while simultaneously setting off an immune-stimulating alarm. The results are encouraging for vaccine development: In both mice, and cynomolgus monkeys, a high-titer polyclonal antibody response was elicited, as well as a cytotoxic cellular immune response.[4] More traditional vaccines that depend on the display of short peptide fragments on the viral coat for antigen presentation are generally not capable of producing such a full-blown immune response: Most often they produce monoclonal antibody responses that require boosters to maintain their viability—and virtually no T-cell response.

Palmenberg thinks these experiments are only the tip of the iceberg when it comes to fusing polypeptide antigens into the recombinant Mengo vector. Her group is working on engineering additional viral cleavage sites into the Mengo genome so that more than one polypeptide copy can be delivered by the recombinant virus. "Engineering this sequence into the vector has a lot of biotech applications that extend beyond vaccines," she says. "Because the ribosome thinks that it is only making one protein as it passes over sequence, a gene therapist could use it to deliver as many genes as Mengo can hold." So far the verdict is out on just how large a genome Mengo can accommodate. But the stability of the virus, after removal of what was initially seen as its poly-C "billboard," indicates there is room for expansion.

Adenovirus-Assisted Transplants

In working with adenovirus, Marshall Horwitz (Albert Einstein College of Medicine, Bronx, NY)

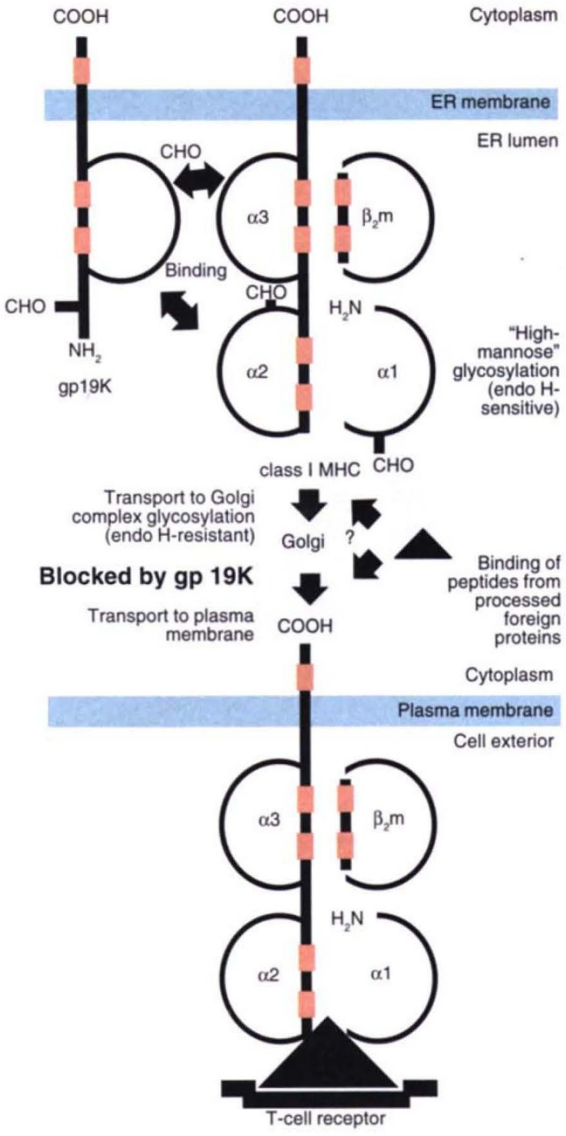

FIGURE 3.
Adenovirus gp19K binds class I MHC in the endoplasmic reticulum, preventing its transport to the cell surface. Recent experiments suggest that gp19K may be used therapeutically to prevent transplant rejection. Adapted from L. Gooding and W. Wold. 1990. *Crit. Rev. Immunol.* **10**:53-70.

recognized what he thought might be a way to convert a viral strategy for immune evasion into a therapeutic application. Adenovirus's E3 region produces a protein in infected cells known as gp19K (see Figure 3). The protein's only known function is to bind major histocompatibility complex (MHC) class I molecules as they make their way through the endoplasmic reticulum—preventing further processing. Horwitz marveled at how efficient this strategy was in preventing immune detection: T-cell killing was significantly decreased because there was little MHC class I available on the cell surface to present viral peptides. Horwitz's first reaction was that this might be an effective tool for controlling transplant rejection.

Horwitz and collaborator Shimon Efrat (Albert Einstein, Bronx, NY) moved quickly to construct a vector that would allow them to test the therapeutic role of E3. "Initially we wanted to make a construct that

would test only the effect of gp19K," says Horwitz, "but technical difficulties convinced us to put in the entire region." Horwitz says that at least six other polypeptides produced by the E3 region may also prove to be beneficial. "Three of these are known to play a role in downregulating tumor necrosis factor's (TNF's) cytolytic function, another protein is involved in the release of virus from the cell, and two others have as-yet-undetermined functions," he says. Hooking up the entire E3 coding region from human adenovirus 2 to the rat insulin promoter, the group then used this construct to generate transgenic mice that expressed the E3 proteins in pancreatic islet cells. Once the mice had reached maturity, these islet cells were transplanted under the renal capsule of allogeneic recipient mice. To the researchers' great satisfaction, these allogeneic grafts survived for more than 90 days with no detectable side effects.[5]

The team is confident that gp19K is the prime mover in allowing these allografted cells to evade immune detection. Although mononuclear cells were found near the allografted islet cells in some animals, suggesting that there was some type of chemotactic gradient attracting them to the site, there was no evidence of T-cell attack. This proposes that whatever attracted the mononuclear cells to the site was not sufficient to launch an immune attack because there was an absence of MHC class I.

Horwitz and Efrat are pursuing experiments that will test possible therapeutic applications of this new E3-based technology. Efrat sees a number of ways to use E3 genes to replace nonfunctional pancreatic tissue in a diabetic patient. "Either islets cells from the donor's pancreas or from standard cell lines might be transfected with the E3 gene to prolong their survival as grafts," he says. "One way to do that would be to keep the E3 gene in the adenovirus vector behind a strong promoter." Gene therapists routinely remove this region when working with adenovirus as a way to increase the vector's gene-carrying capacity: Because E3 is nonessential for growth in tissue culture, its removal allows more genomic space to clone in the gene of interest. But since part of gene therapy's delivery problem results from immune detection of the foreign vector, Efrat and Horwitz see the use of E3 as a way to hold the immune system at bay—giving the gene more time to be expressed—and possibly allowing the vector to integrate into the cells.

For now, the team will only discuss future applications in terms of demonstrating which parts of the E3 gene are necessary for prolonging graft survival. But they are keen on the ability to use discoveries like theirs for new applications. "Viruses have been clever in developing mechanisms to prolong their survival," says Horwitz, "now it is time to exploit this ability to develop novel therapeutics."

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