VIRUSES-The Cornerstone of Gene Therapy

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Abstract

Viruses have given us such dread diseases as smallpox, polio, some forms of cancer, deadly influenza epidemics, the common cold, and AIDS. Yet, these masters of death and destruction are about to pay us back, in full and with interest. For without the viruses, there would be no gene therapy, and if we consider their ancestors, the plasmids, evolution of animal life on this planet would have been a much slower process.

When we speak of curing someone of a genetic disease, we are referring to gene replacement, or the process of introducing a normal gene into a defective cell. But how is this to be done?

These are only a few of the problems that viruses had to overcome as they evolved into cellular parasites. Their first task was to find a way to get their genome into a cell, so the cell's machinery could be used to reproduce their kind. How they managed this, and how their success has become essential for the success of gene therapy, is the aim of this article .

Keywords : Virus, Gene Therapy , Disease, Gene

Somebody once said that a virus is a piece of nucleic acid surrounded by bad news, and when we think of all the trouble these things have caused us over the years, we can easily see the truth in it. Viruses have given us such dread diseases as smallpox, polio, some forms of cancer, deadly influenza epidemics, the common cold, and AIDS. Yet, these masters of death and destruction are about to pay us back, in full and with interest. For without the viruses, there would be no gene therapy, and if we consider their ancestors, the plasmids, evolution of animal life on this planet would have been a much slower process[1].

When we speak of curing someone of a genetic disease, we are referring to gene replacement, or the process of introducing a normal gene into a defective cell. But how is this to be done? Eukaryotes are a clever bunch, and they take a dim view of foreign genes dropping by for lunch.

To protect their privacy, they have surrounded themselves with a membrane that blocks the passive entry of everything except the tiniest of molecules. Even if a piece of DNA could gain access to the cell, it would still have to get into the nucleus before it could be replicated and transcribed. But the nucleus, like the cell, is surrounded by a lipid bilayer that also prevents passive diffusion of anything larger than a water molecule.

Viruses Are Living Crystals

Soon after James Watson and Francis Crick resolved the structure of DNA, Watson published a paper on viral structure in which he suggested that since a virus is a tiny particle, less than one-tenth the size of a bacterium, it could only carry enough nucleic acid for a dozen or so genes. Consequently, he proposed that viral structure must consist of only a few proteins, used over and over again in some sort of symmetrical, highly ordered arrangement. To test this idea, many biologists examined viral structure under the newly available electron microscope, and when they did, they saw tiny crystalline structures that confirmed Watson's speculations.

We now know that most viruses, including the herpesvirus, adenovirus, and poliovirus, have a crystalline protein structure that is icosahedral (constructed from triangles, like a geodesic dome). The protein crystal forms a hollow compartment called the capsid that contains the viral genome. In some cases an envelope consisting of a lipid bilayer, which is often studded with proteins, surrounds the crystalline capsid. Some viruses, such as the influenza virus, have a simple, though highly ordered, spherical capsid instead of a crystalline icosahedron[2].The presence or absence of an envelope, the structure of the capsid, and the nature of the viral genomethat is, whether it is RNA or DNAare the most important characteristics scientists use to identify and classify these organisms.

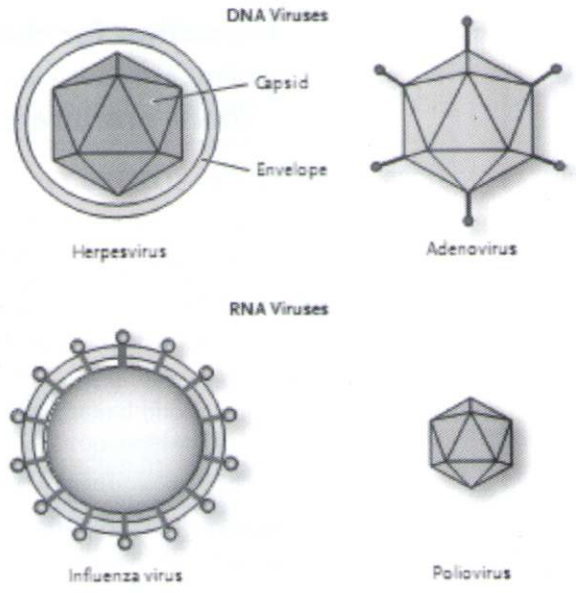

FIG 1 : Viral morphology. Herpesvirus, adenovirus, and polioviruses all have icosahedral capsids, or protein coats, that surround and protect the viral genome, which may be DNA or RNA. Herpes and influenza viruses are also surrounded by a lipid bilayer that may be studded with proteins

Viruses Evolved from Plasmids

It was once thought that because viruses are so simple, they must be extremely ancient and may have been the life-form that gave rise to the prokaryotes. But we know now that all viruses are cellular parasites, incapable of replicating their genome or of synthesizing their proteins without using cellular machinery. Consequently, they must have evolved after cells appeared, and their most likely ancestors are bacterial plasmids.

Plasmids are minichromosomes that bacteria have been swapping amongst themselves for more than 1 billion years. This form of prehistoric neighborly behavior was often of mutual benefit. Plasmids carry antibiotic resistance genes, so if a cell happens to make one that is par ticularly good, another, unrelated bacterium could get a copy simply by capturing the plasmid. Plasmids were probably released into the environment when a cell's membrane became leaky, for various reasons, or when the cell died and broke open, an event that echoes the molecular sharing that may have occurred among the prebiotic bubbles that gave rise to the first living cells. But plasmid exchange among prokaryotes could work only so long as the plasmids stayed small enough to reenter an intact cell by passive diffusion.

The first virus was probably a plasmid that picked up a gene for a protein that could spontaneously form a capsid. Acquiring a capsid made it possible for the virus to interact with cell-surface receptors, some of which are like doorways into the cell, so the virus was no longer dependent on passive diffusion for entry. In this sense, acquiring a capsid was like finding the key to the cell's door. Once the cell-surface barrier was removed, the viral genome was free to increase in size from a few genes to a few dozen.With a larger genome, viruses evolved a wide range of strategies for entering cells and, once inside, taking over cellular machinery to suit their own purposes[3].

The Virus as a Gene Vehicle

Given their talents for entering cells, viruses would appear to be ideal candidates for gene delivery vehicles or vectors. But there are two major Problems to overcome before they can be used safely: First, the ability of the virus to replicate its own genome must be blocked, along with the production of viral mRNA that codes for proteins that maintain the infection and help the virus escape from the cell. Second, the therapeutic gene has to be inserted into the viral genome in such a way that it will not inhibit the formation of a normal capsid, since this is the part of the virus that is essential for cell entry.

Production of viral gene vehicles is carried out in a test tube. Viral genes needed for replication and the maintenance of infection are removed, after which the therapeutic gene is inserted into the viral chromosome. The hybrid chromosome is added to a test tube and mixed with purified viral capsid proteins, leading to the auto-assembly of viral particles. If this is done properly, the virus will be able to enter the cell to deliver the gene, but it will not harm the cell, nor will it be able to reproduce itself.

Viruses Used in Gene Therapy

Adenovirus type 2 (AD-2) and a retrovirus called murine (mouse) leukemia virus (MuLV) have been used in more than 90 percent of all gene therapy trials to date. AD-2, although naturally adapted to infecting the upper respiratory tract, has been used in trials that targeted T lymphocytes, liver, skin, and a variety of tumor cells. An important consideration when using this virus is the amount to give the patient. In a trial attempting to treat a liver ailment, for example, the recombinant AD-2 is injected directly into that organ. If the number of viral particles injected is correct, the liver receptors will bind up all of the viral particles. If the amount is too low, too few cells will take up the virus, so expression of the therapeutic gene will be insufficient to treat or cure the disease. If the amount is too high, viral particles will spill out into the general circulation and infect a variety of cells[4]. Being crippled, these viruses cannot damage the cells they infect, but their presence can lead to a potentially deadly immune response as T cells detect and destroy infected cells. In extreme cases, this can lead to the destruction of entire organs and death of the patient.

While the adenovirus has proved to be a good delivery vehicle, the expression of the therapeutic gene tends to decline after a week or two. This is believed to be due to the extra chromosomal life cycle of this virus. That is, the viral chromosome enters the cell nucleus, but it does not integrate into a host chromosome .Under these conditions, the cell's machinery does not continue transcribing the therapeutic gene .Moreover, AD vectors are inefficient at infecting some cells, and they tend to activate an antivector immune response.

Consequently, many clinical trials have turned to the retrovirus as an alternative vehicle. These viruses are very efficient at infecting cells of the immune system (the AIDS virus has made that perfectly clear) and they do not elicit as strong an immune response as do other vectors.

Moreover, the retroviral life cycle includes integration of its genome into the host chromosome. Once it is in the chromosome, the therapeutic gene is expressed at a steady rate.

Unfortunately, in many cases, the rate at which a therapeutic gene is expressed by a retroviral vector is too low to cure the patient or even toalleviate some of the symptoms. In addition, there is always some apprehension about using an integrating virus, because if something goes wrong, there is, at present, no way to get it out again. This is particularly worrisome, since in an attempt to increase expression of the therapeutic gene, some gene therapy trials use retroviral vectors that are replication competent (can still reproduce).

The justification for designing replication-competent retroviral vectors is that these viruses do not kill the cell when they exit. If its pathology-inducing genes are removed, reproduction of the vector and its movement from cell to cell are of no concern.Vector reproduction leads to an increased number of cells being infected and thus increases the amount of therapeutic protein being synthesized,with subsequent benefits for the patient. However, there is always the possibility that one of these vectors will encounter another virus infecting the patient and, through genetic recombination, become pathogenic and possibly deadly[5].

An alternative approach involves genetic engineering of hybrid retroviruses that might produce large quantities of the therapeutic protein while being unable to replicate themselves. To this end, scientists have recently created an Ebola-HIV viral hybrid to be used as a novel gene delivery vehicle. Scientists know that both viruses are deadly and exceptionally talented when it comes to infecting cells. The hybrid appears to work well in animal experimentation, but whether it will ever be approved for use in human gene therapy trials is another question.Crippled though the vector is, the scare factor associated with it is such that many people will be reluctant to have it injected into their veins.

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