Viruses, Cancer, Warts and All: The HPV Vaccine for Cervical Cancer

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Author: Jacqueline Jaeger Houtman, Ph.D.
Scientific Advisor: T.C. Wu, M.D., Ph.D., MPH, Johns Hopkins University School of Medicine
Scientific Reviewer: W. Martin Kast, Ph.D., University of Southern California

BREAKTHROUGHS IN BIOSCIENCE COMMITTEE
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Mary Lou King, Ph.D., University of Miami School of Medicine
Lorraine Oman-Ganes, M.D., FRCP(C), CCMG, FACMG, American Society of Human Genetics, Toronto, Ontario, Canada

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Science Policy Committee Chair: Mark O. Lively, Ph.D., Wake Forest University, School of Medicine
Managing Editor: Carrie D. Wolinetz, Ph.D., Director of Communications, FASEB Office of Public Affairs
Production Staff: Jennifer Pumphrey, Communications Assistant, FASEB Office of Public Affairs

COVER: The understanding that human papillomavirus (HPV) could cause cervical cancer was the result of decades of fundamental research on wart- and cancer-causing viruses, including the virus from which the legend of the jackalope arose, as well as study of cervical histology. The HPV vaccine to protect against cervical cancer may prevent 70% or more occurrences of this deadly disease. Figures courtesy of Getty Images, Wikipedia Commons, and Jean-Yves Sgro.
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Throughout history, people have been trying to determine the causes of the diseases that plagued them, in hopes of finding a way to prevent or cure them. Some theories seem far-fetched today. The stars affecting influenza, for example, or yellow fever as the result of vapors from rotting vegetation. Or cervical cancer coming from eating bacon and ham.

Today we know that viruses cause all of these diseases, and they can be prevented with vaccines. The newest of these is the vaccine to prevent cervical cancer. This breakthrough involved several converging avenues of basic research—with a few blind alleys along the way.

Cervical Cancer: From Pap Smears to Folklore

Cancer of the cervix—the lower, narrowed part of the uterus—is the second most common cause of death from cancer in women worldwide (Figure 1). It kills 250,000 women per year, mostly in developing countries. In the United States, 11,000 women are diagnosed with cervical cancer each year, and 5,000 women die from it.

Cervical cancer usually develops in a slow progression as normal cells change in size and structure (a condition called dysplasia). Mild dysplasia is considered benign and noncancerous, and usually disappears without treatment. Sometimes, mild dysplasia will progress to moderate or severe dysplasia, and eventually to cancer, which may become invasive and spread to other parts of the body.

The characteristic precancerous changes of cervical dysplasia led George Papanicolaou to propose in the 1940s that cells brushed from the surface of the cervix could be examined under the microscope as a screening test (Figure 2). If precancerous changes are observed, further testing is done and the abnormal
cells can be removed before they develop into cancer (Figure 3). As with most cancers, the earlier it is detected, the earlier it can be treated, and the better the chance for survival or even cure.

The procedure, now known as the Pap smear, has saved countless lives since the 1950s. According to the National Cancer Institute, cervical cancer incidence in this country declined by 74% between 1955 and 1992, due largely to routine screening with the Pap smear.

But the cause of cervical cancer remained elusive. Nuns provided the first epidemiological clues. As early as 1842, Italian physician Domenico Rigoni-Stern observed that unmarried women and nuns in Verona were much less likely than prostitutes, married women, and widows to die of uterine cancer. (In contrast, they were more likely to die of breast cancer, a connection explored in the Breakthroughs in Bioscience article “Breast Cancer, Tamoxifen & Beyond: Estrogen and Estrogen Receptors.”) Fabien Gagnon also found cervical cancer to be practically nonexistent in his study of nuns in Quebec in the 1940s. This suggested that the incidence of cervical cancer might be somehow linked to sexual activity, pregnancy, or childbirth.

Another epidemiological anomaly was reported for Jewish women, who develop cervical cancer at one-twentieth the rate of non-Jewish women. A 1901 report attributed the difference to “the absence of bacon and ham in the diet of Jews.” Later attempts to show a link between cervical cancer and circumcision of male partners or the traditional practice of Niddah (sexual abstinence during and after menstruation) proved controversial. It seemed to be a blind alley (see sidebar, “Necessary, but not sufficient.”)

Exploration of an association with sexual practices and cervical cancer continued, and strong risk factors emerged. The more sexual partners a woman has had and the younger she is when she first has intercourse, the higher her risk for developing cervical cancer. Widows of men who died of penile cancer also have a higher risk, as do second wives of men whose first wives had died of cervical cancer. This epidemiological evidence suggested that cervical cancer was infectious; it could be transmitted from one person to another through sex. The association of cervical cancer with sexually transmitted diseases was a huge clue, but no specific association could be demonstrated with the sexually transmitted bacteria that cause gonorrhea or syphilis.

Herpes was another matter. The genital strain of the herpes simplex virus (HSV-2) is transmitted sexually. In the 1960s and 1970s, evidence began to accumulate that HSV-2 infection was associated with cervical cancer. By 1974, the “herpesvirus hypothesis” was the favored theory among scientists. This theory became the first step towards a vaccine to protect against cervical cancer, but was built on a foundation of nearly a century of scientific discovery.

What is a Virus?

In the 1880s Louis Pasteur and Robert Koch proposed that diseases didn’t arise out of thin air,
but were caused by microbes, and Koch introduced his famous pos-
tulates for determining the cause of infectious disease (Figure 4). Using Koch’s methods, the causative bacteria of anthrax, tuberculosis, typhoid, cholera, pneumonia, gonorrhea, and syphilis were identified. Bacteria could be grown in nutrient broth or on agar plates, forming visible colonies that could then be used to transmit disease. The bacterial cells could be stained and observed through the microscope.

Then there were the troublesome diseases for which the causative bacteria could not be identified. The disease-causing agents could be passed from animal to animal but, unlike bacteria, they couldn’t be grown in culture medium and, when the inoculum was passed through a porcelain filter to remove cells (including bacteria), the cell-free fluid remained infectious. Dmitri Iwanowsky and Martinus Beijerinck independently used the filtration technique to study the mosaic disease of tobacco plants in the 1890s. Beijerinck was convinced that the infective substance was a liquid, which he called “contagium vivum fluidum.”

In the first review of viral disease, published in 1903 in the Bulletin de l’Institut Pasteur, Emile Roux argued that the lower limit of size of the so-called “invisible” microbes should not be limited to the resolving power of the microscopes available at the time. Just because they could not be seen with the microscope didn’t mean they weren’t there. He pointed out the work of Freidrich Loeffler and Paul Frosch, the first researchers to demonstrate that a disease of animals, foot and mouth disease, was caused by one of these “filterable viruses.” Loeffler and Frosch went on to use an even finer filter to remove the viruses, showing that viruses were not liquid after all, but very fine particles, much smaller than bacteria.

An early working definition of a virus was an infectious agent that could not be seen through a microscope, could pass through a filter that retained bacteria, and could not be grown in artificial media. The working definition did little to convey the unique properties of viruses. Unlike other living organisms, viruses are not made up of cells. (There also is some debate as to whether viruses themselves should be classified as living organisms.)

A virus is merely a piece of genetic material (either RNA or DNA), protected by a protein shell. Some viruses have an additional surrounding envelope of lipid-rich membrane, spiked with protein.

Entirely dependent on its host cell for replication, the virus is a study in minimalism. It has all it needs—and only what it needs—to make more of itself. It hijacks the cell’s own machinery to make more of the genetic material and proteins it needs to assemble multiple copies of itself. (Viral replication is described in more detail in a previous Breakthroughs in Bioscience article, “Finding Chinks in the Viral Armor: Influenza, AIDS and Antiviral Therapies.”)

**Virus Hunters**

How do you find something that’s too small to see? At first a virus’s presence could only be inferred by the infectious nature of fluids containing it. In the early days, the only way to demonstrate the presence of a virus was to inoculate it into a susceptible animal and see if the animal got sick. In the 1940s and 1950s, it became possible to grow animal cells in dishes in the laboratory. These cell cultures could then be inoculated with the experimental material. If virus was present, it could cause changes in the cultured cells—they could bunch up or merge into each other or get bigger or smaller, or develop visible clumps inside them. Or they could die.

It wasn’t until 1939 that anyone actually saw a virus. Dr. G. Kausche used the new technology of the electron microscope to produce the first electron micrograph of the tobacco mosaic virus, the agent Beijerinck had called “contagium vivum fluidum.” As scientists trained their electron microscopes on more viruses, they saw a variety of shapes—soccer balls, filaments, bullets, or amorphous blobs, many with protruding spikes (Figure 5). The formerly invisi-
Koch’s Postulates

1. The agent must be present in every case of disease.

2. The agent must be isolated from the host and grown in the laboratory.

3. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy, susceptible host.

4. The same agent must be recovered again from the experimentally infected animal.

Figure 4, Koch’s Postulates: These four criteria are designed to identify a causal relationship between an infection microorganism, like a bacteria or virus, and a disease. In other words, if an infectious agent meets all four requirements, scientists can conclude that it causes the disease that they are studying. Designed by Corporate Press.
ble world of the virus was revealed.

But what if you can’t grow the virus and you don’t have an electron microscope handy? Another way to find a virus is to look for its proteins, using antibodies, a component of the immune system. If an animal is infected with a virus, its immune system will make proteins called antibodies. Antibodies can bind specifically to a virus and neutralize it or help the immune system eliminate it from the body. After infection, the anti-viral antibodies remain to protect against future infection. Because antibodies bind so specifically, they are a powerful scientific tool. The antibodies can be purified and tagged, with a fluorescent marker, for example. When the tissue in question is treated with the tagged anti-viral antibody, the virus glows under fluorescent light.

Yet another way to find a virus is to look for its genetic material. The genes of each virus contain a specific sequence of bases. By using a synthetic probe with a complementary sequence, you can find the viral sequence. The probe is labeled with a radioactive tag. If the viral sequence is present in the target tissue, the target sequence and its complementary probe will stick together, like two sides of a zipper. Because the probe is radioactive, it will produce a spot on photographic film, allowing the researcher to see it. This is called DNA hybridization, and it was the key to finding the cause of cervical cancer (Figure 6).

Can a Virus Cause Cancer?

Determining the cause of a disease is much more difficult with viruses than with the bacteria that led Koch to propose his postulates. For one thing, viruses are more likely to be host-specific; a chicken virus won’t grow readily in a guinea pig, for instance, or a human virus won’t grow in a mouse. This makes it difficult to find an appropriate experimental host to use in place of a human. Viruses also need living cells in which to multiply, and finding suitable cells in which to grow viruses in the lab can be difficult. Finding a viral cause for cancer required more flexible thinking and creative study, especially when most scientists were resistant to the idea that a virus could cause cancer.

In 1908, Danish scientists Vilhelm Ellerman and Oluf Bang were the first to isolate a cancer-causing virus. The avian leukemia virus causes leukemia in chickens, but since leukemia was not considered to be cancer until after 1930, little notice was taken.

Peyton Rous also studied chickens, but he focused on solid tumors called sarcomas from Plymouth Rock chickens (Figure 7). He ground up the tumors and transplanted the extract to unaffected chickens, producing tumors. It had already been established that tumor cells can
1. Using biochemical techniques, scientists are able to isolate the genetic material (DNA and RNA) from tissue. If a virus is present, the DNA or RNA of that virus will be isolated, too.

2. Double stranded DNA is "unzipped," usually by applying heat, to expose a single strand of nucleotides or bases (the building blocks of DNA) for a probe to bind to. This is called denaturing.

3. A radioactively labeled probe is mixed with the denatured DNA. This probe is based on the sequence of bases from the virus being sought.

4. If the viral DNA is present, the probe will bind to the complementary sequence. This is known as hybridization.

5. The DNA is transferred to a filter or other solid material. It is then exposed to a piece of photographic film. If the virus is present and the probe has been hybridized, the radioactive label will leave a dark spot on the film. This is called an autoradiograph.

Figure 6, DNA Hybridization: Designed by Corporate Press
cause cancer when transferred from animal to animal, so Rous went further. He filtered the cells out of the tumor extract, using a filter fine enough to exclude both tumor cells and bacteria. When the recipient chickens developed tumors from the filtered extract, Rous concluded that the new tumors were caused by something other than cells, something smaller. In the 1911 paper describing his findings, he wrote, “The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism. Analogy with several infectious diseases of man and the lower animals, caused by ultramicroscopic organisms, gives support to this view of the findings.”

Controversy surrounded Rous’s discovery for at least 15 years. People were so resistant to the idea that a virus could cause cancer that they came up with reasons why Rous was wrong. Some claimed that it wasn’t really cancer, but some kind of inflammatory response to the virus. Others claimed that it was a phenomenon unique to chickens, with no relevance to human cancers.

We now know that cancer-causing (oncogenic) viruses are not limited to those that infect chickens. Oncogenic viruses have been found in mice, cats, dogs, and even frogs. The first human virus demonstrated in 1965 to cause cancer was the Epstein Barr virus, (EBV). EBV is most commonly thought of as the cause of infectious mononucleosis (“mono”), but it has also been associated with several rare forms of cancer. Currently, there are six viruses recognized as potentially oncogenic in humans (Table 1).

By the mid 1970s, it was accepted that viruses can cause cancer, and evidence began to mount implicating HSV-2 in cervical cancer. HSV-2 and its DNA had been found in association with cervical cancer cells, and women with cervical cancer often have antibodies to the virus in their blood, evidence of HSV-2 infection.

Like all herpesviruses, the genetic material of HSV-2 is made up of DNA. The DNA is surrounded by a protein coat, which is in turn surrounded by a protein-studded membrane. Marek’s disease, a cancer of chickens, had been shown in 1967 to be caused by a herpesvirus, and the human her-

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Virus Name</th>
<th>Associated Disease</th>
<th>Type of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillomaviridae</td>
<td>Human papillomaviruses (HPV)</td>
<td>Warts</td>
<td>Cervical cancer, cancer of the skin, anus, penis</td>
</tr>
<tr>
<td></td>
<td>Kaposis’s Sarcoma herpesvirus</td>
<td>Cattleman’s disease</td>
<td>Kaposis’s sarcoma</td>
</tr>
<tr>
<td></td>
<td>Epstein-Barr virus</td>
<td>Mononucleosis</td>
<td>Burkitt’s lymphoma, Nasopharyngeal cancer, Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>Human T-lymphotropic virus (HTLV)</td>
<td>Tropical spastic paraparesis</td>
<td>Adult T-cell leukemia/lymphoma</td>
</tr>
<tr>
<td>Hepadnaviridae</td>
<td>Hepatitis B</td>
<td>Hepatitis, liver disease</td>
<td>Liver cancer</td>
</tr>
<tr>
<td>Flaviridae</td>
<td>Hepatitis C</td>
<td>Hepatitis, liver disease</td>
<td>Lymphoma</td>
</tr>
</tbody>
</table>

Table 1. Known oncogenic (cancer-causing) viruses in humans.
pesvirus, EBV, was also implicated in several kinds of cancer. It seemed plausible, then, that HSV-2, also a herpesvirus, could cause cervical cancer. Data supporting this hypothesis were presented at the 1974 Cervical Cancer meeting in Key Biscayne, Florida.

But German virologist Harald zur Hausen had been unable to replicate the HSV-2 results. At the Key Biscayne meeting, he presented his findings that HSV-2 was not found in cervical cancer. His pronouncement was met with stunned silence. Surely zur Hausen’s techniques were just not sensitive enough.

In fact, zur Hausen believed that a virus was associated with cervical cancer, but he thought HSV-2 was a blind alley. HSV-2 was a first step towards a vaccine, but it was a step in the wrong direction. Since he couldn’t find evidence of a link with HSV-2, he had his eye on a different virus...the wart virus.

**Jackalopes, Warts and Papillomaviruses**

A wart is a benign overgrowth of cells on the skin (where it is also known as a papilloma) or mucous membranes. Warts afflict rabbits, sheep, cattle, horses, dogs, monkeys, and deer, as well as people. They can spread between people and from one part of a person’s body to another. Warts may occur anywhere, but commonly affect the hands and feet, or the genital area, where they are also known as condylomas. Warts have been around since ancient times and their cause has been attributed to such diverse activities as washing hands in water in which eggs have been boiled, killing a toad, or masturbation. The infectious nature of warts has long been suspected in animals and humans and several anecdotal reports appeared in the nineteenth century. In 1907, an Italian researcher named Cuillo inoculated his own hand with a filtered wart extract, producing warts and demonstrating their infectious capacity.

More thorough study began in the 1930s. Richard Shope was already a virologist of some repute, having discovered the first influenza virus (in pigs) in 1931. He had heard stories of wild cottontail rabbits that had “horns” or growths on their heads and other parts of their bodies. It is thought that these rabbits gave rise to the legend of the “Jackalope” (Figure 8). He obtained some of these warts from a hunting buddy in Iowa and ground them up with a mortar and pestle. Then, as Rous had done, he filtered out the cells. He used a needle or sandpaper to damage the shaved skin of unaffected rabbits and applied the wart extract. Within six to twelve days, they had warts, too. The virus was named the Shope papillomavirus (later renamed cottontail rabbit papillomavirus or CRPV).

In 1949, Maurice Strauss of Yale University published the first electron micrographs of papillomavirus particles in skin warts. Unlike herpesviruses, papillomaviruses have no lipid envelope. The outer shell of the human papillomavirus is made up of proteins put together in the
shape of an icosahedron, so it looks a little like a soccer ball (Figure 9). Inside the virus’s shell is genetic material made up of DNA.

**Papillomaviruses and Cancer**

The evidence was strong that HPV can cause warts. Cancer was another matter, but zur Hausen had reasons for suspecting papillomaviruses in cervical cancer. In the 1930s, Richard Shope had observed that domesticated rabbits infected with his papillomavirus often developed warts that progressed to cancer after about four months. Shope was too busy at the time to pursue that line of research, so he passed the materials to Peyton Rous. Rous published his findings with Joseph Beard in 1935; a rabbit papillomavirus can cause warts that become cancerous (Figure 10).

Chickens and rabbits were one thing. But what about a link between papillomaviruses and cancer in humans? Evidence for the involvement of papillomaviruses in human cancers was provided by the studies of the Stefania Jablonska at the Warsaw School of Medicine and Gérard Orth at the Institut Pasteur in Paris in the 1970s. They studied the very rare skin disease called epidermodysplasia verruciformis and demonstrated HPV was present both in the wart-like growths and the cancerous growths that sometimes arise on areas of the skin exposed to sunlight. This was the first direct association of HPV with cancer in humans.

Papillomaviruses can cause warts that become cancerous in rabbits and humans. Papillomaviruses were also shown to cause cancer in cows and sheep. Genital warts provided another clue, since they are caused by sexually transmitted papillomaviruses. Both HSV-2 and human papillomaviruses had epidemiological connections with cervical cancer, but zur Hausen’s group could not demonstrate the presence of HSV-2. Could sexually transmitted papillomaviruses cause cervical cancer?

**Obstacles Faced by Papillomavirus Researchers**

Harald zur Hausen could hardly have picked a more difficult virus to study. He seemed to face obstacles at every turn.

**Obstacle One: Species specificity.** Papillomaviruses are highly species specific. That means that you have to use rabbit papillomaviruses in rabbits. It also means that there is no laboratory animal that can be infected with human papillomaviruses.

John Kreider and his colleagues at the Milton S. Hershey Medical Center in Pennsylvania got around that obstacle and came up with a way to grow human papillomaviruses in mice. Nude mice have defective immune systems because they have no thymus. They also have no hair (hence the name). Because of their defective immune systems, nude mice don’t reject tissue transplants from other animals. In the 1970s, Kreider grafted rabbit skin onto nude mice and was able to grow rabbit papillomaviruses.

Kreider then turned his attention to human papillomaviruses. He transplanted normal human cervical tissue (obtained from women
who had had hysterectomies) or human foreskin tissue (obtained from newborn circumcisions) near the kidneys of nude mice. The grafts were infected with extracts of precancerous cervical tissue. The grafts showed changes typical of precancerous cervical lesions and, perhaps more importantly, served as a source of infectious virus. William Bonnez and Robert Rose from the University of Rochester later adapted the technique to use SCID mice, which have a more severe form of immune deficiency, and have become one of the best animal models for studying basic biology of the immune system, providing insight into a host of diseases, including cancer.

**Obstacle Two: The viral life cycle.** Nobody could seem to get papillomaviruses to grow in cell cultures. The reason for that, as it was later determined, is that the life cycle of papillomaviruses is intimately tied to the differentiation of the cells they infect. The complexity of the cellular requirements for virus growth just couldn’t be replicated in a lab dish, at least not at first.

The surfaces of the skin and mucous membranes are covered with a layer of cells called epithelium. In normal epithelium, cells in the deeper, basal zone multiply and then change in structure and appearance as they migrate toward the surface, becoming flatter as they move through the intermediate zone. At the surface, in the superficial zone, the cells eventually die and are sloughed off (Figure 11).

Papillomaviruses first infect cells in the basal zone (requiring a tiny scratch or abrasion, called microtrauma to reach the basal zone). The virus multiplies along

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**Infected Epithelium**

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**Figure 10. Peyton Rous:** Despite the resistance to his early work with cancer in chickens, Rous continued to study cancer. In 1966, he won the Nobel Prize in Physiology or Medicine, 55 years after he isolated the sarcoma virus that now bears his name. He shared the prize with Charles Huggins, who had investigated the role of hormones in cancer (See the Breakthroughs in Bioscience article, "Breast Cancer, Tamoxifen & Beyond: Estrogen and Estrogen Receptors.") Image courtesy of the National Library of Medicine.

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**Figure 11. HPV in the epithelium:** Epithelium is the tissue made up of layers of cells that line the structures and internal cavities throughout the body. HPV infects the basal (bottom) layer of epithelium and produces "early proteins" which cause cell division. As the epithelial cells divide, they carry the viral genes with them. The top layer of epithelium is sloughed off, and the lower layers rise through the intermediate zone to the top or superficial layer, becoming more differentiated. When this happens, the virus in the infected cells begins producing "late proteins" which are involved in virus capsid production and assembly. HPV is then released with the sloughed off, infected cells. Adapted from Koss et al., *Introduction to Gynecologic Cytopathology with Histologic and Clinical Correlations*, 1999.
with cells in the intermediate zone. Infected cells carry the virus DNA within the cell, but separate from the cell’s own DNA. As the cell divides, it makes copies of both its own and the virus’s DNA, which is passed along to the daughter cells.

In addition to the genes that code for proteins that make up the virus’s shell, the papillomavirus has genes for two groups of proteins, called early and late proteins. Early proteins are present in the cell during active infection, but they are not present in the mature virus particle. Early proteins help make copies of the viral DNA and also stimulate the cells in the basal zone to divide, and to continue dividing even when they migrate to the intermediate and superficial zones. Because infected cells are stimulated to multiply, the epithelium thickens, producing a wart.

The late proteins, made in the intermediate zone, make up the outer shell of the virus. They are the only proteins that are included in the virus particle itself, which is assembled in the superficial zone and released when the cells are sloughed.

It wasn’t until 1992 that infectious HPV particles were produced in lab dishes. A specialized type of cell culture called organotypic or “raft” culture produced differentiating cells in which papillomaviruses would grow. The process involves growing the cells on a porous collagen gel, then raising the cells up to where the growth medium meets the air above it. This technique produced infectious virus particles, but not a lot of them.

**Obstacle Three: A lack of infectious virus in cancer.** As Richard Shope had demonstrated in 1933, infectious virus can be isolated from warts. Linking HPV to cancer, however, proved difficult, in part because in the process of making a cell cancerous, the viral DNA incorporates itself into the cell’s DNA, and it is no longer possible to assemble infectious virus particles (see sidebar “How do papillomaviruses cause cancer?”). Since he couldn’t look for infectious virus in cancer tissue, zur Hausen had to find the virus another way. He looked for viral DNA.

At the time, DNA hybridization was the method of choice for identifying specific DNA sequences. Warts were ground up and the virus particles were purified in the lab. DNA was isolated from the purified virus and a complementary probe was made from the purified DNA. The probe was then used to test DNA from other warts for the presence of the matching viral DNA sequence (see Figure 6).

**Obstacle Four: Not enough virus in warts.** Many warts didn’t yield enough virus particles to isolate the DNA. The problem was overcome as molecular biology technology advanced. Investigators could now insert viral DNA into circles of bacterial DNA (plasmids). The plasmids multiplied along with the bacteria, providing ample viral DNA to make all the probes they needed for hybridization and other studies.

**Obstacle Five: Viral types.** Probes corresponding to known types of HPV wouldn’t bind to tissue samples under the usual conditions unless the match was perfect. When conditions were changed so that the match didn’t have to be perfect, the probes would bind, suggesting that there were more viruses out there that were similar, but not identical to
those that had been characterized to date. This was, at first, a cause of great consternation.

As DNA hybridization studies proceeded through the 1970s, it became evident that there were several distinct HPV types that differed in their DNA sequences. As the types began to be sorted out and numbered (in order of discovery), it also started to become evident that certain types of HPV were associated with particular types of warts, for example, some are usually associated with common hand warts, while others are more often found associated with plantar warts on the soles of the feet.

The first HPV DNA zur Hausen isolated was from genital warts in 1980, then from a laryngeal papilloma in 1982. These were HPV6 and HPV11, respectively. But they were not found in any cervical cancer tissue. Then zur Hausen's student, Mathias Dürst, changed the DNA hybridization conditions to isolate the first HPV sequence from cervical cancer cells. In 1983, he wrote, "The data reveal a startling prevalence of this DNA in malignant tumors and its very occasional presence in benign papillomas." This virus was named HPV16, and it was detected in about half of the cervical cancer biopsies. Another papillomavirus, HPV18, is present in about 20% of cervical cancers.

Today, more than 100 types of HPV have been isolated. Most of them are "low-risk." They may cause warts, but are highly unlikely to cause cancer. There are at least 15 "high-risk" HPV types that have been implicated in cervical cancer, but HPV18 and HPV16 are by far the most prevalent.

Because working with papillomaviruses themselves proved to be so difficult, researchers relied on molecular techniques and worked with the viral DNA instead. As newer techniques became available, obstacles to working with papillomaviruses could be overcome, or at least sidestepped. DNA technology was the key to finding the virus and discovering its heterogeneous nature. It was also revealing the secrets of how HPV causes cancer (see sidebar, "Necessary, but not sufficient"). In fact, the DNA alone is enough to turn cultured cells into cancer cells. HPV DNA has been found in cell lines derived from human cervical cancer cells and a worldwide survey found that HPV DNA is present in more than 99% of cervical cancers. DNA analysis is reveal-

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**Necessary but not sufficient...**

Yet another difficulty in proving that HPV causes cervical cancer was that the vast majority of HPV infections do not result in cancer. Most HPV infections are cleared by the immune system. Persistence puts woman at higher risk, and is required, but even if the viral DNA takes up residence, it usually doesn't result in cancer. HPV is a necessary link in the chain of events leading to cervical cancer, but it is not the only one. Other factors, both environmental and genetic, may affect the progression to cancer in persistently infected cells.

It is known, for example, that women who smoke are at a higher risk for cervical cancer. The cancer-causing chemicals present in cigarette smoke make it more likely that cellular mutations will occur in HPV-infected cervical cells, making it more likely that they will become cancerous.

Despite its fall from favor as the causative agent for cervical cancer, HSV-2 may turn out to play a role after all. Unlike HPV, HSV-2 is not a necessary step in the progression to cancer, but it seems to increase the risk. HSV-2 proteins may act as cofactors in cervical cancer progression and could promote the transformation of HPV-infected cells to invasive cervical cancer. HSV-2 as a cofactor in cervical cancer progression remains a controversial idea, but it just one of many potential environmental factors that may play a role in the development of cervical cancer.

Another apparent blind alley in the search for a cause for cervical cancer was the observation that Jewish women had a low risk of cervical cancer. New findings suggest that the reasons for that decreased risk are genetic, and not cultural. Molecular analysis has shown that many Jewish women possess a protective genetic variation in the p53 gene that could affect the way this tumor suppressor protein interacts with HPV early proteins. Although these findings, too, are controversial, they may provide an insight into the genetic factors that may be required for cervical cancer to develop.

By studying the environmental and genetic factors that contribute to cervical cancer in HPV-infected cells, scientists are learning more about this and other cancers. By revealing the causes, they may develop new ways to prevent or cure a variety of cancers.
ing just how intimately HPV and cervical cancer are linked.

**Where There’s a Virus, Is There a Vaccine?**

Vaccines prevent disease by exposing the immune system to an innocuous form of a disease-causing organism. The immune system responds by producing cells and antibodies that recognize that organism. When the actual organism is then encountered, the immune system is ready, and can rid the body of the organism before it has a chance to cause disease.

In 1796, Edward Jenner inoculated a boy named James Phipps with the harmless cowpox virus to protect him from the related but deadly smallpox virus. Since then, vaccines have saved millions of lives. Just one man, Maurice Hilleman, is credited with saving more lives than any other scientist in the 20th century by developing eight of the fourteen most commonly used human vaccines, including those against mumps, measles, rubella and meningitis (Figure 12). Hilleman developed the first ever vaccine against cancer—the Marek’s disease vaccine, introduced for use in chickens in 1972. He is also credited with developing the first ever vaccine against human cancer. The hepatitis B vaccine, introduced in 1981, was intended to prevent a form of viral hepatitis, but has had the added benefit of preventing the liver cancer that sometimes results from hepatitis B virus infection.

![Figure 12, Maurice Hilleman: Often called the "godfather of vaccines," microbiologist Maurice Hilleman developed more than three dozen vaccines, including the first vaccines against cancer. He once said "Science has to produce something useful. That's the payback to society for support of the enterprise." Hilleman certainly lived up to that statement: as the developer of vaccines against mumps, measles, rubella and meningitis, to name only a few, he is credited with saving millions of lives. Image courtesy of the National Library of Medicine.](image)

Could a vaccine be produced to protect against HPV infection, or even cervical cancer? The evidence suggested that it might be possible. As far back as 1937, Richard Shope had observed that rabbits that recovered from papillomavirus infections were immune to reinfection. He had also vaccinated uninfected rabbits with wart extracts so that they became resistant to infection.

Another piece of evidence was that cervical cancer is more likely to occur in women whose immune systems are defective or suppressed. That suggested that the immune system might somehow hold HPV infection in check and play a role in preventing cervical cancer from developing.

With high hopes, Harald zur Hausen approached pharmaceutical companies with a proposal to develop a vaccine against HPV in 1984. They all turned him down.

**An Empty Shell of a Virus**

Harald zur Hausen’s dream of a vaccine to prevent cervical cancer wouldn’t die, but a safe and effective vaccine for HPV could not be produced in the usual way. The most common types of vaccines use a killed or weakened version of the infectious agent to stimulate the immune system into recognizing and fighting the real thing more effectively. If this kind of vaccine was made for HPV, it would contain the viral DNA. Since viral DNA alone is enough to cause cancer, at least in the laboratory, any kind of vaccine that contained HPV DNA would pose an unacceptable risk.

Another approach, which has been used successfully for the hepatitis B vaccine is a subunit vaccine. Subunit vaccines contain just a part of the virus, not the whole thing. Early attempts at papillomavirus subunit vaccines used purified L1, the major structural protein, with disappointing results.

In the early 1990s, four groups took up the zur Hausen’s challenge: Douglas Lowy and John Schiller at the National Cancer Institute; William Bonnez and Robert Rose at the University of Rochester; Richard Schlegel and Bennett Jensen at Georgetown University; and Ian Frazer and
Jian Zhou at Queensland University in Australia. All four groups were looking for ways to make an HPV vaccine. The way was opened with genetic engineering.

Researchers had come to rely on genetic engineering to work with a virus that refused to grow easily in the laboratory. They made multiple copies of HPV DNA to study. They could also insert viral DNA into bacteria, and into yeast, insect, and mammalian cells. Those cells could then crank out viral proteins. When they cranked out the main structural protein of HPV, called L1, something interesting happened. Five copies of the protein arranged themselves into viral subunits called pentamers, 72 pentamers then arranged themselves into what looked like a virus. The 360 puzzle pieces of protein had self-assembled into a “virus-like particle,” or VLP.

Here was a potential vaccine that should be safe. It contained no DNA, so it could not multiply or cause cancer. VLPs looked just like the real virus under the electron microscope (Figure 13). They could fool the eye, but could they fool the immune system?

**Does it Work?**

The fact that HPV can only grow in human cells means that the animal efficacy trials must be done with the animal equivalent of the vaccine. Again, investigators relied on the biological effects of nonhuman papillo-

![From HPV to VLP to Vaccine](image)

Figure 13. From HPV to VLP to Vaccine: Virus-like particles (VLP) consist of structural proteins from the outer capsid of the virus. They resemble the viruses from which they are derived but, because they do not contain any genetic material, are not infectious. However, the VLP’s mimicry of the virus is enough to elicit an immune response strong enough to protect against viral infection and disease. The HPV vaccine is one of the first VLP vaccines. Image designed by Carrie Wollenetz and Corporate Press.

maviruses in nonhuman models to form the groundwork for HPV study. Species-specific versions of the VLP vaccines were tested in rabbits, dogs, and cows. The vaccinated animals produced high levels of antibodies and the vaccines were at least 90 percent effective at preventing experimentally induced warts. The good news was that antibodies in the blood of vaccinated animals could protect uninfected animals from infection, indicating that the antibodies elicited by the vaccine were sufficient to protect against infection. The bad news was that the vaccines were type-specific, meaning that they could only protect the animal against the papil-
lomaviruses of the same type as the vaccine VLP. This tempered expectations for the human vaccine, since more than one HPV type can lead to cervical cancer.

VLPs of human papillomaviruses were first tested in monkeys to see if they could induce antibodies. They did, but monkey trials could not assess protection afforded by the vaccine, since monkeys can not be infected with HPV. The ultimate test for VLP vaccine effectiveness would be clinical trials.

Of course, it would be unethical to wait until cancer develops to see if the vaccine can prevent cancer, so the clinical trials looked at safety, antibody levels, viral persistence, and early, precancerous changes. Clinical trials showed that the vaccines induced high levels of type-specific antibodies against HPV. Women vaccinated in the trials were also protected from persistent HPV infection and precancerous cervical changes.

The first HPV vaccine was approved by the FDA in June of 2006. It is called Gardasil® and is produced by Merck using VLPs produced in yeast cells. It is quadrivalent, containing VLPs from four different HPV types: the two most prevalent high-risk viruses, HPV 16 and HPV18, as well as the two most common causes of benign genital warts, HPV6 and HPV11. GlaxoSmithKline’s bivalent vaccine, called Cervarix®, contains VLPs for HPV16 and HPV18. The Cervarix® VLPs are produced in insect cells. This vaccine is currently awaiting FDA approval. By preventing persistent infection and precancerous changes, the vaccines will theoretically prevent cervical cancer developing in 10 or 20 years.

**Future Directions**

The current vaccines are effective against HPV16 and HPV18, the two types that together account for about 70 percent of cervical cancer cases. Unfortunately, the vaccines seem to be relatively type-specific, protecting primarily against the VLP types included in the vaccine. There are at least 13 other types that account for 30 percent of cervical cancer cases. The likelihood that the current vaccines will protect against all these other types is slim, so new vaccines must be developed that will provide a broader spectrum of protection. In addition, work continues to make the vaccines more stable and cheaper, so that they may be used in developing countries where the majority of cervical cancer occurs.

The HPV vaccines are effective when administered to women or girls who have not yet shown evidence of HPV infection. They are not effective at treating women who have already been infected with the virus, which is a sexually transmitted agent. Therefore, the National Institutes of Health recommends the vaccine be given before an individual is sexually active. Research is underway to produce vaccines that may provide protection if administered later in the cancer causing process. Meanwhile, Pap smears remain an important part of protection against cervical cancer, in both vaccinated and unvaccinated women.

It took a twisted road full of blind alleys, unlikely intersections, and seemingly insurmountable obstacles. Through creative thinking and persistence, the path was found and obstacles were overcome—or bypassed—to reach ultimate success: a vaccine with the potential to save hundreds of thousands of lives every year.
Biographies

Jacqueline Jaeger Houtman, Ph.D. writes about biomedical science from Madison, Wisconsin. She enjoys writing for physicians, scientists, middle school students, and the general public. She has written for The Dana Foundation, World Book, and scientific journals, including Clinical and Experimental Allergy and Journal of NeuroVirology. She can be reached at jjhoutman@nasw.org.

T.C. Wu, M.D., Ph.D., MPH, is a professor in the Departments of Pathology, Oncology, Obstetrics and Gynecology and Molecular Microbiology and Immunology at Johns Hopkins University Medical Institutions. His laboratory focuses on developing vaccines and immunotherapeutic strategies for the prevention and treatment of human papillomavirus (HPV)-associated cervical cancer. Dr. Wu has published extensively in this area, holds a number of patents and has been recognized for his work by the International Academy of Pathology, the Passano Foundation, and the Society of Chinese Bioscientists in America.