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VIRAL VACCINES IN HUMANS

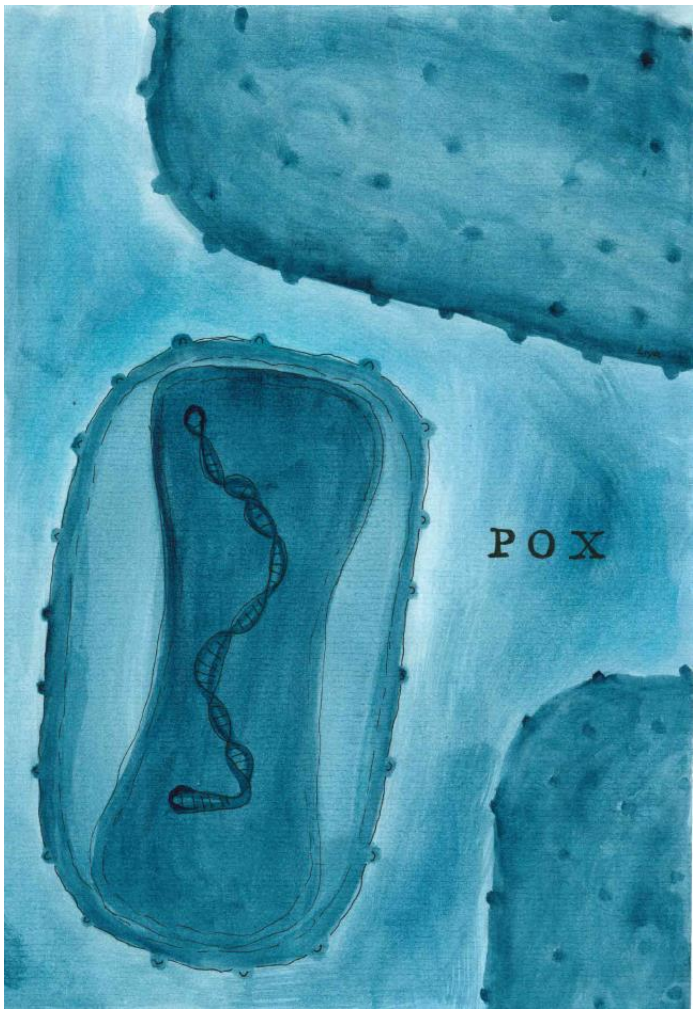
Britta Wahren, Microbiology, Tumor and Cell Biology, Karolinska Institutet

Illustrated by Liva Herlenius, Photo Lennart Nilsson.

Every year several animal viruses jump over the species barrier and become new human infections. In the fight against viral infections, smallpox and wild type polio are eradicated or almost eliminated. Hepatitis B virus (HBV) and some Human Polyoma viruses (HPV) have been drastically reduced, and its related tumours have, as well. Endemic influenzas, and several herpes viruses are in need for vaccines covering their subtypes and mutants, while Covid-19 caused by Sars-CoV-2 has become an endemic infection despite effective vaccines. No effective vaccines have been found against human immunodeficiency virus (HIV) or hepatitis C virus (HCV), but antiviral drugs convert HIV to a chronic state and other drugs can eliminate HCV. There are many concepts for successful vaccine treatments to which Karolinska Institutet in Sweden has contributed during the last 80 years.

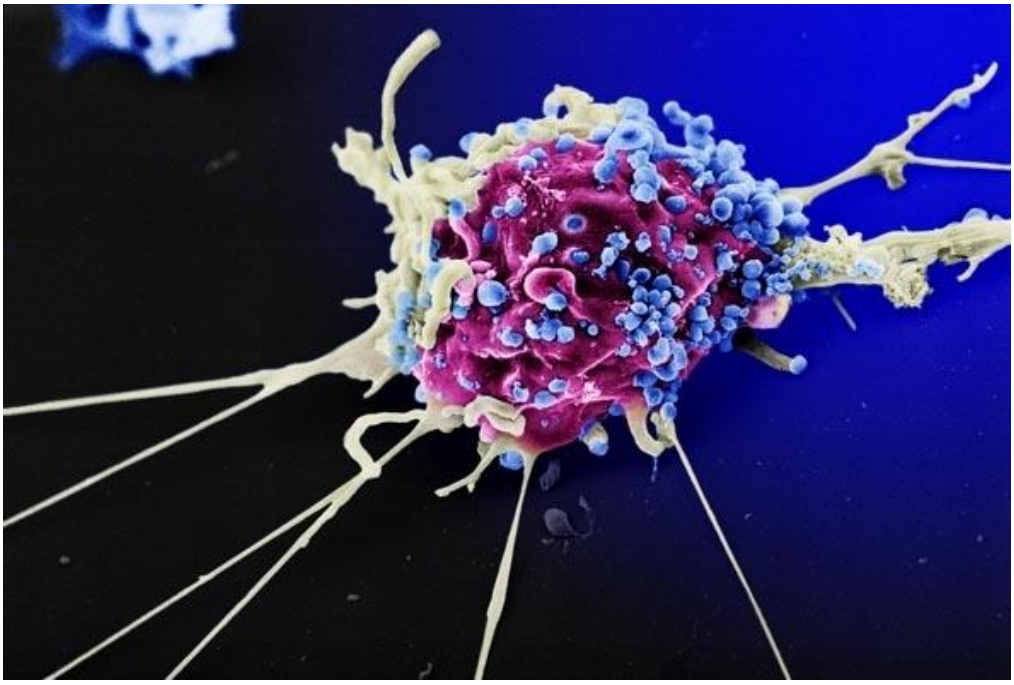
Smallpox was eliminated from people, but the virus is contained in freezers.

Smallpox, caused by the variola virus, had plagued humanity for centuries, causing widespread death, disfigurement, and social disruption. Hundreds of millions of people have been left permanently scarred or blind by this disease. The smallpox disease spreads easily through contacts, causes fever, multiple pocks and had a death rate of 10-30 percent, depending on the infecting strain.



Pox viruses are our largest DNA viruses, and they multiply only in the cytoplasm of cells (Figure 1. Pox virus). The double-stranded DNA of pox viruses can code for 150-300 different proteins, around which around 75 make up the mature virion. The photo of a cell infected by the pox virus vaccinia was taken by **Lennart Nilsson**, a renown photographer who often worked at Karolinska Institutet. Late in his career he installed not only one but three electron microscopes in various cellars, among them the Microbiology Tumor and Cell Biology department. His picture shows a cell infected by vaccinia, a variola-related

pox virus. The cell is shrinking and slowly dying, while producing myriads of blue virus particles (Figure 2. The dying cell. Photo Lennart Nilsson).



The disease is characterized by hundreds of pocks in the middle of the body, in contrast to varicella – chicken pox, which mostly gives rise to pocks on the extremities.

Efforts to combat smallpox intensified with the development of an effective vaccine by Jenner in 1976. The World Health Organization (WHO) led an eradication campaign that started in the 1990-ies.

The smallpox vaccine used for eradication contains the whole virus particle of vaccinia virus, which protects against several members of the pox virus family. Immunization started with the rather unsophisticated pocks forming on the skin of sheep. The pock´s content was scraped into glass tubes and used to pick into human skin by bifurcated needles. Later cleaner tissue cultured virus was concentrated and used. Stocks of vaccinia vaccine are held by most countries, in preparation for an eventual sudden outbreak. The attenuated virus strain Modified Vaccinia Ankara (MVA) became the preferred vaccine due to its non-toxicity. The MVA strain of vaccinia has also been frequently used as a vector for other virus gene fragments.

Holger Lundbäck was the director at the Swedish National Bacteriological Laboratory (SBL) during 1962-1982. A part of SBL preceded the department of Microbiology Tumor and Cell Biology at the Karolinska Campus in Stockholm. He participated in the eradication of smallpox through his work at WHO. He was also a member of the Global Commission for the eradication of smallpox and of the Expanded Program on immunization in the Global advisory group of WHO. In these capacities he took responsibility for elimination of the very last cases of smallpox.

Countries across the world joined in the vaccine effort to eradicate smallpox. The United States and the Soviet Union worked together through the darkest cold war days; and professionals from more than 70 nations served as WHO field staff. On October 26 in 1976, the last case of smallpox occurred.

During 1816-1976 it was a legal obligation to be immunized against smallpox in Sweden. Although smallpox is now eradicated from the human population, the virus remains in two laboratories, in Atlanta, US and in Novosibirsk, Russia.

Ring immunization consists of immunizing all contacts found around a case, whether healthy or sick. By this methodology it was finally possible to eradicate smallpox. This task is easy when you can identify a case by the appearance of pocks on the skin. It is more difficult but was still useful in the case of an epidemic of Ebolavirus infections in several countries in West Africa in 2014 with a vaccine built on a vector of the vesicular stomatitis virus.

In Sweden, smallpox contributed to the death of over 300,000 individuals between 1750 and 1800. Our last outbreak occurred in Stockholm in 1963, causing infection of 27 persons and 4 deaths. It was caused by a traveller, mistaken to have a varicella infection.

The reasons that smallpox vaccine is effective is due to the existence of an immunogenic and stable vaccine and the lack of animal carriers. A third reason is, that it was easier to spot people with pox eruptions on the skin, than it is to identify persons with for instance airway infections.

Mpox derives from rodents.

Monkey pox (mpox) is a viral infection spread mainly through close physical contact with an infected person. Physical sexual contact poses a particularly high risk. Common symptoms are blisters, fever, and swollen glands. It is usually a mild self-limiting disease, but immunodeficient persons may experience a more severe disease.

Monkey pox virus was first identified in captured monkeys but in the wild it occurs in rodents in several countries in central Africa.

The vaccine given for mpox is the attenuated vaccinia vaccine, which is the same vaccine as that given against smallpox. The disease became more frequent at the same time as the effects of the worldwide immunization to smallpox waned, indicating that cross-reactivity had been induced against monkey pox by the vaccinia-virus vaccine.

Mpox occurs endemically in western and central Africa. The disease has however increased lately, and cases have spread to the US and Europe. A small outbreak occurred in 2003. Some people in the US who had contact with pet prairie dogs appeared with pox lesions. It was found that the prairie dogs had been housed with small rodents imported from Ghana.

A new series of mpox infections occurred in 2021 after two persons travelled from Nigeria to the US. There is still an ongoing slow spread of the mpox disease in 2022-2024 in Europe and the US, mostly through sexual contacts. The campaign with information and vaccination in risk groups has significantly reduced the spread. The disease is not as deadly as smallpox variants were, but it is still important to immunize persons with immunodeficiency diseases that might be at risk for sexually transmitted disease. Still cases of mpox persist in Sweden.

There are three wild polio strains, of which two have been eliminated.

Polio, a small RNA virus, presents with symptoms ranging from general malaise to paralysis, and in severe cases, it can lead to death. Polio viruses exist as three different serotypes 1, 2 and 3 (Figure 3. Polio virus). Polio viruses have a sturdy outer coat, the capsid. It is made by 20 even-sided triangles to form an icosahedron. This makes them retain infectivity in various environments. The virus primarily spreads through water contaminated with faeces, a pathway that was not fully understood until in the mid-20th century. This lack of knowledge contributed to its widespread global transmission. Recognizing the importance of hygiene in disease prevention, Nordic countries generally fared better in managing polio outbreaks.

Sven Gard, head of the Department of Virology at Karolinska Institutet, led efforts to develop a Swedish polio vaccine. The feasibility to culture virus in cells made it possible to produce large amounts of virus vaccines. For this invention **John Enders**, **Thomas Weller** and **Frederick Robbins** received the Nobel Prize in 1954. The technology provided means for culturing, multiplication, and concentration of pure virus particles into vaccines. There were different theories of whether such a vaccine should be attenuated live or killed for safety. **Jonas Salk** advocated an inactivated vaccine and **Albert Sabin** an attenuated oral vaccine, while Sven Gard chose to inactivate the Swedish polio vaccine by formaldehyde. The killed vaccine used in Sweden contains all three polio types, but the immune response against each type can vary.

Mass vaccination campaigns commenced in 1957, and by 1962, the polio cases in Sweden had significantly declined. By 1977, Sweden's success in eradicating polio led to its declaration as polio-free by the World Health Organization.

The Global Polio eradication program initiated in 1988 predicted total eradication by world-wide vaccination by 2020. However, wild type 1 polio persists in Afghanistan and Pakistan. Unfortunately, in a few cases an attenuated strain, particularly of polio type 1 can survive the oral-fecal route and be transmitted continuously. Mostly, the spread of such strains is due to poor vaccine coverage. Wars, general mistrust, and the spread of other pandemic viruses have interrupted the completion of polio vaccine campaigns.

In addition, vaccine-derived polio strains of varying pathogenicity have occurred in many countries. The attenuated polio vaccine has until today been used for primary vaccination of

children in developing countries due to the ease of mucosal immunization by drops in the mouth. In large parts of the world attenuated strains of the polio virus types 1, 2 and 3 were therefore used as oral vaccines. Attenuated vaccine strains have then unfortunately emerged and spread by the fecal route in countries like Pakistan and Nigeria. Killed vaccines can be used for boosting the primary immune responses induced by attenuated live polio vaccine.

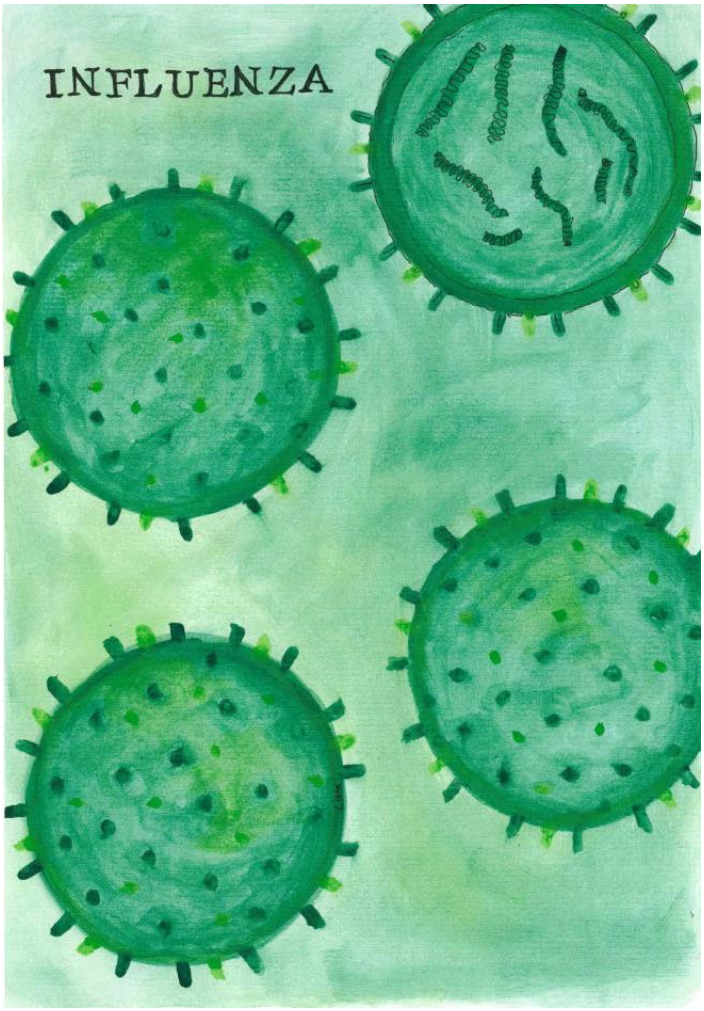
An incident in the Cutter laboratory in the US in 1955 led to distribution of polio vaccine lots that were not completely inactivated. 260 children got infected with polio, and 11 children died. Generally, such occasions led to caution to vaccinate with whole virions or attenuated virus strains, despite the general efficacy of such vaccines.

New types of vaccines are being developed with inserted mutation mutants in sites of replication. Such man-made recombinant vaccines are now used (2024) to end the spread of vaccine-derived polio strains.

Airborne viruses are the most contagious ones.

Influenza epidemics

Influenza virus spreads continuously, mostly by birds. In humans it manifests itself as an acute respiratory infection with fever, sore throat, muscle pain and headache. The virions occur as subtypes A, B and C. They can easily vary, due to their fragmented RNA genes (Figure 4. Influenza virus). The RNA influenza virus particles are very vulnerable due to their fragile fatty outer membranes. Therefore, the particles are easily inactivated to make a vaccine. Today generally only subparts of the virion membrane, the hemagglutinin and possibly neuraminidase glycoproteins are given as a split vaccine. Not until the 1940-ies both A and B strain hemagglutinin proteins could be incorporated into a vaccine.



The ordeal for making a vaccine that fits the circulating virus strains depends on the origin of the virus. Wild birds are the main reservoirs and transmit to swine which in turn are natural carriers of several other subtypes. Humans are susceptible to infections from both species.

WHO has a special group that surveilles flu viruses in 114 countries around the world. As soon as the strains frequently occurring in humans in the southern hemisphere are analyzed by the WHO

laboratories in the region, the preparation for the composition of this years´ influenza vaccine is made. Generally, this happens in February and the vaccines are ready to be injected in the Northern hemisphere in December the same year. Three or four strains are the common composition. This year (2024-25) two A strains plus one B strain will be found in the flu vaccine.

The efficacy of the yearly influenza vaccination has generally been around 50 percent against notable infection, 80 percent against mortality.

As state epidemiologist at the Swedish Institutes for Infectious Disease Control (SBL, SMI and FHM), **Annika Linde** took a large part in monitoring and analyzing influenza in Sweden. She introduced the sentinel system, consisting of groups reporting influenza disease and strains from various parts of the country. During Linde's tenure, national surveillance of influenza was intensified and structured, with a focus on capturing data on severe and fatal cases. Enhanced reporting mechanisms, including weekly surveillance reports were developed. The members of the European Influenza Surveillance Network (EISN) are part of WHO.

The previous most severe outbreak was the Spanish flu in 1918 which was caused by a very virulent A strain. This influenza virus killed up to 50 million persons, mostly in Europe.

Trials to find and use a less variable site like the stem of the hemagglutinin protein as a vaccine has been made with satisfactory duration of group-specific immunity.

The very first genetic vaccine for humans consisting of DNA plasmids encoding Georgia influenza A was designed by **Margaret Liu** (Hon Dr Sci at Karolinska Institutet) and collaborators at the Merck company. Clinical studies in Baltimore showed a moderate induction of antibodies to the flu hemagglutinin. These data were not very impressive but preceded the coming explosion of DNA and mRNA gene constructions made for use as vaccines.

There is also mRNA-based injectable vaccine produced as non-replicating or self-amplifying genes, saRNA. The two latter constructions permit a much lower dose of vaccine since the influenza RNA gene amplifies itself intercellularly for a limited period.

In the veterinary field there are diseases of the so-called A-type, which should be eradicated as soon as detected. H5N1 influenza belongs to these dangerous viruses. It caused a small but very lethal human outbreak in Hong-Kong in 1997 and thereafter disappeared. Now H5N1 viruses have circulated in birds in Europe including Sweden since 2019. It has also contaminated other birds, seals and dolphins around the coasts of South America, polar bears, skunks and most recently even cows in the US. From cows it has in a few cases directly infected humans. The H5N1 viruses are epidemic among certain animals in several parts of the world. If the H5N1 once again infects humans, this may be our next pandemic.

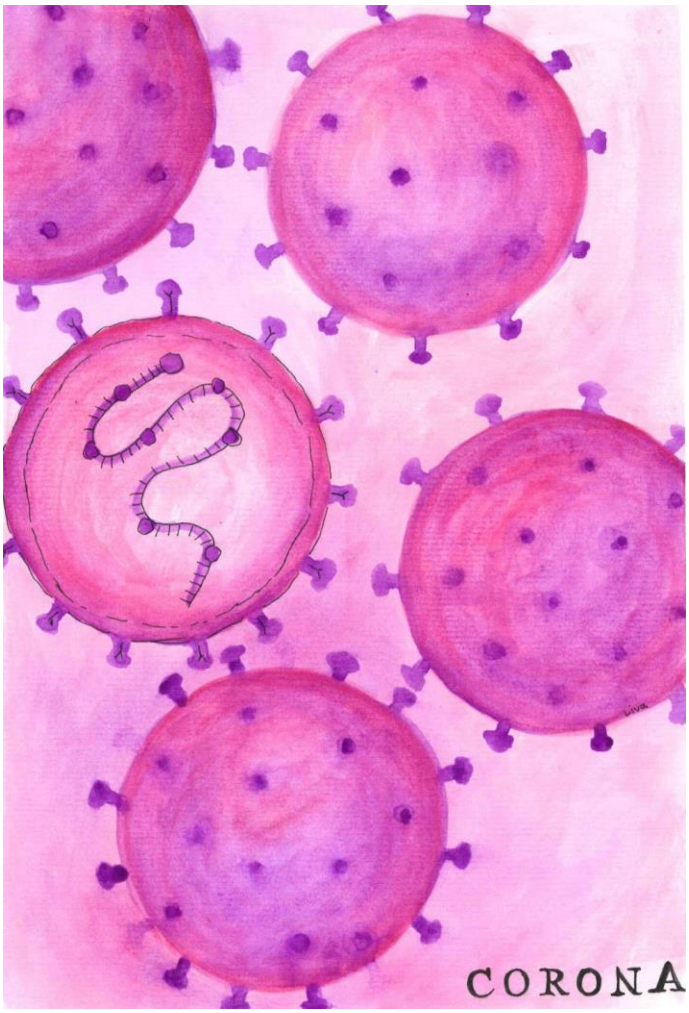
When we look ahead, there is a need for better prediction of coming circulating influenza strains. However, neither predictive antibody measurements nor attempts at sequence predictions have been sufficiently accurate.

Corona viruses of varying pathogenicity

Other airborne viruses are the RNA corona viruses. In November 2002 a particularly lethal strain of coronavirus SARS (severe acute respiratory syndrome), started to spread locally in China/Guangzhou and Hongkong and spread by air passengers to Canada and other countries. It derived from civets in meat markets and had a lethality of over 10%. Due to its short incubation

period and massive disease, it could be managed by virus isolation around cases without any vaccine. The disease disappeared two years later. Today there are experimental vaccines against the Sars-1 virus infection.

A severe acute respiratory syndrome MERS (Middle East respiratory syndrome) appeared among camel handlers in Saudi Arabia in 2012. The infectious agent was traced to bats and camels, which do not succumb to the infection. An intense pneumonia leads to a mortality rate of 30%. Quarantine measures contained the disease. Today there are still a few human cases, traced to raw camel milk. A vaccine has been developed, intended for camels. MERS remains an epidemic/pandemic threat if mutating in the carrier bats to strains that spread more easily in humans.



In late 2019, the world witnessed the emergence of the novel coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), causing the infectious disease known as Covid-19. The infection caused by SARS-CoV-2 appeared late 2019 in Wuhan and is still - in 2024 - circulating world-wide with continuously mutating strains (Figure 5. Corona virus). SARS-CoV-2 virus primarily causes severe pneumonias, but due to its common main receptor ACE2 (angiotensin-converting enzyme 2), located at cell membranes of cells in many organs, may cause several other organ malfunctions.

The rapid spread during 2020 prompted WHO to announce a pandemic state, which endured March 2020 to May 2023. Luckily a new Omicron variant of SARS-CoV-2 appears to have higher binding for the epithelial cells in the upper respiratory airways, thus usually causing lesser disease than the original Wuhan strain.

Remarkably fast the genome of the first strain was sequenced in Wuhan, and the results were shared world-wide. This led to an exceptional race to produce vaccines, won by companies that decided to use the then novel mRNA technology. It stimulated vaccine production world-wide, and within one year, two mRNA vaccines against the Wuhan Covid-19 strain were used for mass vaccination in several countries. In parallel, vaccines based on inactivated virus particles as well as vector-born genes were developed.

A special type of mRNA vaccine is based on self-amplifying RNA (saRNA) derived from the genome of RNA viruses, mainly alphaviruses. They encode a viral replicase in addition to the antigen. The replicase can amplify the saRNA in transfected cells, reducing the amount of genetic

material needed for vaccination. These novel findings have led to approval of such vaccines by the national drug agencies of India and Japan.

In 2022 **Katalin Karikó** and **Drew Weissman** received the Nobel Prize for enabling mRNA to remain stable and immunogenic during immunization of humans.

When the virus started to spread in countries outside China, the mortality from respiratory malfunction was high in elderly individuals. Rare genetic variants in human defence genes such as the interferon pathways, can lead to severe disease. This was the case in over 30% of unexpected critical disease of Covid-19 infections in healthy young persons. A febrile activity began at KI to study the immunology, collect samples, and to develop rapid polymerase chain reactivities to detect viral genomes. Several groups now study immunology as a basis for humoral and cellular protection.

Immunization of several cohorts of immunocompromised patients with an mRNA-based vaccine revealed that cancer patients responded well while patients with common variable immunodeficiency responded poorly. This would indicate that donation of specific immunoglobulins would benefit the latter patient group. Monoclonal antibodies have been developed which have specificity against the viral spike protein and inhibit virus infection of cells. When given to immunodeficient individuals as an intravenous or intramuscular injection early during infection, such antibodies react with the virus and can inhibit the infection. The effect is, however, short term and there is no remaining effect in immunodeficient individuals.

The recent Covid-19 pandemic has given rise to over 500 million cases from simple coughs to multiorgan failure world-wide, and over 6 million deaths (WHO 2023).

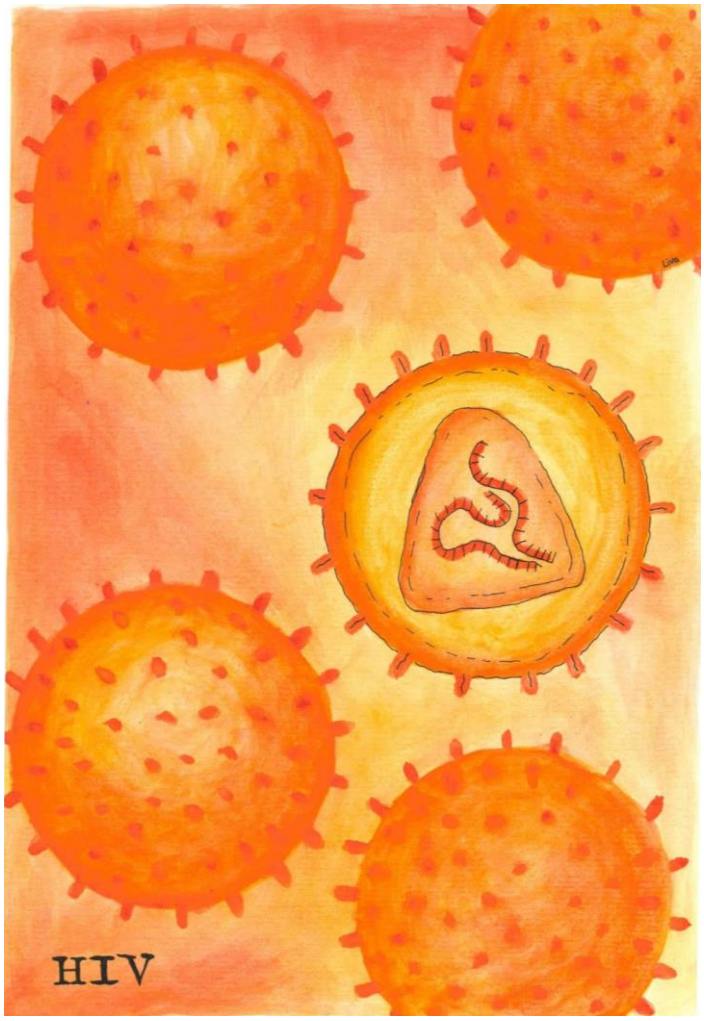
Retroviruses integrate in the host genome, persist life-long and might be cancerogenic.

Retroviruses are also RNA viruses, but not airborne. They are however eternal passengers in the infected body by incorporation of DNA in the genome of the cells they infect. In 1975, Nobel laureates **David Baltimore** and **Howard Temin** revolutionized virology by identifying reverse transcriptase, an RNA-dependent DNA polymerase, changing the central dogma of DNA-RNA-protein synthesis. **George Klein** and his group at the department of Tumor Biology had described the common nature of virus-induced tumours, in contrast to the unique antigenic composition in chemically induced tumours.

The AKR murine retrovirus causes leukemia. These leukemias are hereditary, which means that leukemia develops in adulthood of sensitive mouse strains. Early on around 1960 it was shown by **Britta Wahren** and **Eva Klein** that vaccination with either the whole tumor cells or the purified virus delayed or eliminated the induction of leukemia by the AKR Gross retrovirus. Also, the development of leukemias in mice caused by the Friend virus could be prevented by vaccination, either by tumor cells from the spleen or by cell-free lysates with viruses from the tumors. The vaccine effect derives from both common virion antigens and transformed cell surface antigens, which may vary between transformed cells. Virus-specific antigens are present both in the virions and in the virus-transformed tumor cells.

Human immunodeficiency virus (HIV), a hereditary retrovirus, resides in lymphoid CD4+ cells and leads to acquired immunodeficiency syndrome (AIDS) in the infected, non-treated individual (Figure 6 HIV, human immunodeficiency virus). Since it is mainly carried in lymphoid cells, not

in the gametes, the transfer to a newborn can be stopped by various means, such as a cesarian section or antiviral drug treatment of the mother. The affinity of the virus for the lymphocyte CD4 and CCR5 receptors means that virus persists and is carried around to most organs in the body where it causes symptoms of immunodeficiency and finally AIDS.



For the identification of HIV as the cause of AIDS and immunodeficiency **Luc Montagnier** and **Francoise Barré-Sinoussi** received the Nobel Prize in 2008.

Many, many researchers have attempted to develop protective or therapeutic vaccines to HIV, but there is yet no product that is close to licensing. It was early predicted that vaccine efforts would be difficult, and this is still true today.

The large variability of the HIV virus posed challenges for vaccine development. A novel technology using multiple DNA plasmids, representing various HIV sub-strains and genes, were designed, and shown to induce both antibody and T-cell responses. To produce vaccine protein, the DNA plasmids must be delivered intracellularly. Innovative techniques like gold bullet-mediated delivery of vaccine plasmids, jet air shots, and electroporation were used to enhance the intracellular delivery of the HIV-DNA nucleic acid to humans.

Clinical programs initiated in Sweden, Tanzania, and Mozambique utilized this plasmid vaccine, which was produced at the Vecura laboratory at Karolinska Institutet. Several clinical HIV vaccine programs were initiated in Sweden, Tanzania, and Mozambique by **Eric Sandström**, **Gunnel Biberfeld** and **Britta Wahren**. The latest phase 3 efficacy study of HIV vaccines for prophylaxis failed to show efficacy, like several previous HIV vaccine studies. On the contrary anti-retroviral drugs have shown a strong prophylactic effect against HIV infection.

Despite progress in antiretroviral drug treatment, HIV-infected children are still born due to the inability to eradicate the virus post-infection. Immunotherapeutic vaccination aims to reduce viral burden during antiviral drug treatment.

Maternally HIV-infected children do not respond immunologically to the HIV virus infection. Therefore, immunotherapeutic HIV vaccinations are made to reduce the viral burden during antiviral drug treatment. Efficacy in these cases would be no rebound or over 3 months to rebound following a drug therapy interruption. Such vaccine studies in HIV-infected children are ongoing

in Cape Town together with Karolinska Institutet, University Tor Vergata and the Bambino Jesu Hospital in Rome, and the Henry Jackson Foundation, in Washington DC.

Prophylactic vaccines are good, immunotherapy is lacking.

Vaccines to infectious diseases have reduced child mortality world-wide by over 50% thanks in large part to vaccinations. War conflicts and epidemics such as the recent Covid-19 pandemic make vaccinations more difficult. The schedule for children in Sweden is continuously modified to combine several virus and bacterial antigens, preferable given together at fewer occasions. Viral vaccines given to children are directed against measles, mumps, rubella, rota, polio, and later sometimes human papilloma viruses and hepatitis B virus. Bacterial vaccines are mainly directed against diphtheria, tetanus, pertussis, and Haemophilus influenzae B as well as pneumococci. Vaccine against tuberculosis, Bacillus Calmette-Guérin, is given only if needed, and if a skin test for the same bacterium is negative. Protein vaccines against the parasite Plasmodium falciparum malaria are at a developmental stage.

The two most successful vaccines have contributed to the reduction of tumors induced by these viruses. Hepatitis B virus (HBV) vaccines contain the virus surface antigen HBsAg. The vaccine was developed by **Maurice Hilleman** at the Merck company around 1980. It protects against liver infection and thus also against liver tumors induced by this virus.

The prediction of HPV causing cancer resulted in a Nobel Prize to **Harald zur Hausen** in 2008. Vaccines to the tumor-inducing subtypes have been distributed world-wide. **Lena Dillner** and **Joakim Dillner** at the Karolinska Institutet have demonstrated that widespread use of the HPV vaccine interferes with infection and tumorigenesis.

There are many new concepts for successful vaccine treatments to which researchers at Karolinska Institutet have contributed. A vector based HIV vaccine in preclinical stage was developed by **Peter Liljeström** and his group. He pioneered the Semliki Forest virus vector, which has shown excellent adjuvant immunogenicity for several virus vaccines. Adjuvating by vectors or combination with antivirals may be the best immunotherapeutic approach for difficult viral diseases, as well as virally induced tumors.

Matti Sällberg at the Huddinge campus developed DNA-based vaccines against Hepatitis C virus (HCV). His efforts have involved the development of novel vaccine candidates targeting different components of the HCV virus, including enzymatic genes/proteins and conserved epitopes essential for viral replication. Cytotoxic T lymphocytes and sometimes antibodies were raised but the virus could not be eradicated. Both invariant and variant proteins/peptides or nucleic acids with or without adjuvants have also been used against HCV. They induce HCV-specitic cellular responses but still without clearing of the virus. Direct-acting drugs in combination with vaccines might be one way to go for difficult to treat HCV-infected individuals.

The inactivated whole virion rabies vaccine stands out as a successful intervention, particularly when administered before or within a week after exposure during the virus's three-week incubation period. This immunotherapeutic vaccine approach usually involves the simultaneous administration of large quantities of neutralizing antibodies alongside the vaccine.

It is still an enigma, why it is seldom possible to revert an early virus infection by immunotherapy. Immunotherapeutic strategies for treating chronic HIV, HBV or HCV infections have so far met with limited success.

Organizations monitoring infectious disease and vaccines.

The World Health Organization (WHO) keeps track of infectious diseases and other urgent health issues worldwide. Within WHO, various subgroups focus on specific infections like influenza and Covid-19. They also provide reagents to help with diagnostic work and publish manuals on drug composition, good laboratory practices, and good manufacturing practices. WHO works closely with health institutes and universities globally, including the Public Health Agency (FHM), the European Centre for Disease Prevention and Control (ECDC), and the Swedish Medicines Agency (Läkemedelsverket) in Sweden.

The Bill and Melinda Gates Foundation co-funded the Global Alliance for Vaccine Initiative (GAVI), which focuses on providing vaccines to children and adults in the poorest countries. Thanks to GAVI, millions of lives have been saved from diseases like measles, malaria, and tuberculosis. Currently, they are focusing on vaccines for Covid-19, Ebola, and Dengue. New vaccines for Chikungunya are also being developed, including a live attenuated vaccine and a pox vector-based vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI) was established in 2017 by public, private, and philanthropic organizations to develop vaccines as close to the final product as possible. Their headquarters are in Oslo. CEPI focuses on diseases that might cause sudden outbreaks in humans, such as Nipah, West Nile virus, Chikungunya, Zika, H5N1 influenza, and new coronaviruses. They have developed vectored Nipah strains that protect animals and have several vaccine candidates for various types of influenza. They are also working on vaccine cassettes for any new virus or microbe, tentatively called disease X. Their goal is to develop new vaccines within around 100 days using these vectors with any new insert.

A Global Vaccine Library has been proposed to serve as a repository of data and near-ready vaccine cassettes for the various virus families that may cause the next outbreak.

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Further reading with main message.

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