Viral infections: better understood, better contested

VIRGO
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Introduction

Virgo has made a huge step forward in the rational approach to virus control. The success of vaccines in the past was often due to trial and error, but this strategy is no longer always effective. We need more knowledge about viruses to develop a clearly focused approach and better vaccines.

Within Virgo, we have applied and developed new tools in order to create this knowledge. We have focused on technology for the discovery of new viruses and on research into the virus-host interaction, while looking closely into the cycle as a whole – from the infection by a virus up to and including the host’s response at the molecular level.

Of our twenty thousand genes, a quarter is involved in the immune system; if you want to know exactly what is going on, you should not only consider one cog in the wheel, but rather take a stance that allows you to see the big picture. The genomics technology that we are using now already offered this possibility, but had not yet been used to the full in virus research. Nowadays, genomics is one of the many tools in this field.

Hundreds of millions of people are still infected by dangerous viruses each year. Our genomics approach is a step forward, but it is also a great challenge: it provides a wealth of infinitely complex data.

Presented here is a book describing the groundbreaking results that have been achieved within the Virgo LSH-FES consortium. LSH stands for ‘Life Sciences & Health’, or rather the application of life sciences to the benefit of our health, and FES for the ‘Fund for Economic Structure Enhancement’, financed by the so-called natural gas profits. Virgo started its activities in 2010 upon the Dutch government’s approval of a joint proposal, which was submitted by a combination of many partners. These included small and large businesses, multinationals, academic and medical research groups, patient organisations, and the ministries of Health, Welfare & Sport, Education, Culture & Science, and Economic Affairs.

The FES grant was used to establish a public-private partnership: every single euro invested by the partners was matched by the government. After six years of hard work, the results can be directly applied in the treatment of patients or used for research that is closely linked to patients.

The consortium has thus proven that it has used the research funds to contribute to the improvement of our national public health and boost economic activity in the Netherlands. A vast number of these results are presented in this book. The most recent developments in this exciting field will astonish you time and time again. I therefore trust that you will greatly enjoy reading this book!
Follow the immune response step by step

Eradicating a virus is not as easy as it seems. While viruses used to be isolated within small host populations, our contemporary lifestyles make it far easier for them to reach the entire world’s population. Vaccines do not always help. Research consortium Virgo is seeking a better approach to viruses: when you know exactly how they work in the body, you can treat infections more successfully or even prevent them.

“Viruses are smart”, Virgo Coordinator Ab Osterhaus says. Although they cannot reproduce themselves, their parcels of genes suffice for them to be multiplied by a host and spread from there on. Since they rapidly mutate, viruses can easily take advantage of niches. This makes them quite difficult to control. The changing world has actually made life easier for viruses. Osterhaus: “When I graduated, the smallpox virus had just been eradicated. At the time, the idea was that we would soon be able to manage all viruses this way. This, however, was far too optimistic. In fact, we are faced with growing numbers of infections and almost all of them stem from the animal world.” Viruses then jump from the animal host to humans, with major consequences sometimes. In a small, isolated human population, a virus has far more difficulty to keep going, simply because there are not enough hosts to survive. Osterhaus: “Due to intensive cattle breeding as well as the global village effect, viruses are able to spread more quickly than ever before.”

Over-response

In order to tackle this problem Virgo focuses on various antiviral strategies. “The best thing, of course, would be for us to make a vaccine”, coordinator Arno Andeweg says. Great success was achieved in the past with vaccines of cultured viruses that had been attenuated or killed, but could still lead to an immune response that protected the individual against the real virus. “Many of these vaccines were discovered by trial and error, but unfortunately that approach is not suitable for all viruses. For RSV, a virus that causes respiratory infections in young children, such a vaccine actually triggered an over-response, causing the body itself to further damage the lungs.” Therefore, greater knowledge of the host response and the regulation of this response is essential in order to develop effective vaccines in a rational way.

Pestering

Virgo is applying new techniques to connect this knowledge. By meticulously examining all the steps of the virus-host interaction, Virgo scientists are able to determine exactly when and where to tackle the virus. When a virus enters a host, the immune system is triggered immediately – with a quarter of our more than twenty thousand genes jumping into action. T-helper cells, for instance, put other immune cells to work, B-cells are prompted to make antibodies, and cytotoxic T-cells to kill cells infected by the virus. At the DNA level, all sorts of things happen in these cells; genes are switched on and off in order to clear up the infection and are transcribed in messenger RNA. Protein synthesis and other biochemical intermediary and final products also change under the influence of a viral infection.

The Virgo research projects focused on all these levels. This had led to a range of biomarkers: indicators that help towards the diagnosis, treatment and prognosis of an infection. For example, if a biomarker shows that someone is highly sensitive to a certain viral infection, you are able to predict that the infection will be severe and so give the patient more targeted treatment. The technology has also helped to discover new viruses, such as the MERS virus. In such cases, the strategy is to collect as much data of the unknown infection as possible in order to detect similarities with known viruses and develop a treatment method. And, finally, this approach can result in new or improved vaccines, since a host’s response to a vaccine can now be measured more accurately.

“Naturally, it is impossible to prevent all viral infections”, Andeweg adds. New viruses continue to appear, time and time again. Some viruses that were virtually eliminated, such as measles and polio, still prove capable of raising their head again – for instance due to a low degree of vaccination in regions of crisis. “The pestering is not over yet. We’re still playing a cat-and-mouse game.”

Virgo focuses on four groups of viral infections which, together, comprise the most common viruses. The first group is the acute respiratory viral infections, including influenza, SARS, MERS and RSV. Viruses causing diarrhoea (acute enteral viral infections), such as the Norovirus, are the second group. The third group includes the chronic liver infections hepatitis B and C, and the fourth is HIV.
Immune system model improved by cleverly combining data

Whether or not a person falls ill after contracting a virus partly depends on how capable their immune system is of fighting off the virus. However, parts of the mechanism used to fight off viruses are still unknown to geneticists. Bioinformaticians of the Virgo research consortium are investigating which genes play a part in this process.

Both viruses and their hosts profit from being able to recognize each other – viruses because they are able to reproduce on their own, and hosts because they are able to fight off intruders if they are unaware the intruders are intruders. They recognize each other through proteins, whose blueprints are stored in the genetic materials of both the virus and the host.

Keeping a tally of genes

Virologists are trying to get a good understanding of what happens when a virus and a host interact, as this will help them fight viruses. However, it is hard to determine what actually happens at the genetic level. “If you’re studying the measles virus, you mainly learn to what extent the virus is involved. You need to perform a ‘weighted’ tally.”

After solving many statistical riddles, Van der Lee and Huynen ended up with a collection of a few hundred genes. A new challenge followed, in that they now had to verify that all these newly found genes were actually involved in the human immune system. “This was an immense amount of work”, Van der Lee states. As part of the study, Utrecht University virologists conducted a number of experiments. In this way, the bioinformaticians arrived at a model which, according to their calculations, presented a much more accurate description of the immune system than the current model.

Intervention strategies

This sophisticated model offers clues which may help scientists come up with new intervention strategies to fight infections in the future. However, for now the research is of a more fundamental nature: we now know that many more genes are involved in organizing our defence against all sorts of pathogens.

Discovering gene functions by combining data

Scientists derive new knowledge from combining and analysing data sets. But where to get the data sets?

Protein interaction

Study databases of measured interactions between human proteins and virus proteins. Chances are that such human proteins (and therefore the genes associated with them) are involved in the immune system.

Gene expression

When investigating different types of infections, compare the levels of gene expression in infected persons and virus-free persons. If you find different levels of activity, you have identified genes which are involved in the immune system.

Genome regulation

Some proteins stimulate cells to read DNA. Quite a few of these “transcription factors” are known to play a part in the immune system. If you then determine which part of the DNA they “match”, you will know which gene they activate.

Genome evolution

Since virus-host interaction is a matter of “staying one step ahead of the other party” for both the virus and the host, genes which play a part in the immune system tend to evolve more quickly than other genes. If you compare human genetic material with genetic material obtained from apes and other organisms, you can identify the main differences.

Genetic data

Genetic material obtained from several sections of the human population can be compared. Immune system-related genes show greater variety than other types of genes since genes in different parts of the world are exposed to different viruses.
Virus killers: a great economic success too

The Virgo consortium has yielded a kaleidoscope of new insights, diagnostic tools, vaccines and spin-off companies. “It has more than recovered the investments made by FES, the Economic Structure Enhancing Fund”, says Ab Osterhaus, Professor of Virology.

The Virgo project has certainly given virology a boost in terms of new knowledge and techniques. New technologies have helped prevent pandemic viral infections, and viral mysteries – like the Trojan horse that delivers the measles virus – have been unravelled. So much more is known now as to how viruses invade the body, which receptors they use as a key to enter a cell and how they reprogramme host cells in order to multiply themselves. Dozens of new viruses have been discovered. Virgo coordinator Ab Osterhaus explains: “We are now able to identify all genetic material in, for instance, fluid from the lungs of seriously ill patients through deep sequencing, and then to use bioinformatics tools to determine which virus is causing the disease. The new techniques enable us to quickly recognise a virus like the MERS coronavirus as ‘new’, which means we can also develop diagnostics, medication and a vaccine sooner.”

Top publications
The increase in knowledge can more or less be quantified in publications and doctoral theses as well as in numbers of newly discovered viruses, developed vaccines, patents, and spin-offs (see frame). This project has produced dozens of publications in scientific journals, for example, including a great number of articles in top-ranking journals like Science and Nature. Some researchers have obtained their doctoral degree in this Virgo project and have flown the nest in order to apply their knowledge to new virological issues all around the globe. The Rotterdam Viroscience Lab has grown into a Centre of Excellence.

Self-supportive
The FES funds were not only intended to promote scientific success, but also to encourage cross-pollination with the world of business. “We have calculated that the FES investment has been paid back ninefold in subsidies from outside the Netherlands and funds from the industry. In other words, we have injected new money into the Dutch economy”, says Eric Claassen, initially involved in the project as Professor of Immunology and now Professor of Entrepreneurship in Life Sciences at VU University Amsterdam. “The FES money was the starter engine. However, such funds are only useful if they lead to something sustainable, so we specifically wanted to instate a research group that could pay its own way. We started out with just the six of us. With the ten million grant from the FES funds, we eventually created a hundred jobs in applied biomedical research for the next ten years – a thousand men years of work. This earned us the Valorisation Award of one million euros from the Netherlands Genomics Initiative (NGI).”
The sniffling dromedary and the deadly virus

It was a death in Saudi Arabia caused by an unknown virus that signalled the start of a test case for the Virgo toolbox of genomics, proteomics and bioinformatics for the discovery of viruses, development of vaccines and prevention of spreading.

Summer 2012
A sixty-year old patient of the Egyptian doctor and virologist Ali Mohamed Zaki died in a Saudi hospital. The patient had pneumonia and kidney failure. Zaki had managed to cultivate the virus and suspected that the man had an infection with a paramyxovirus, from the same family as measles and mumps.

The group led by Ron Fouchier at the Erasmus University Medical Center had recently published a new diagnostic method for such viruses. Zaki contacted Rotterdam and sent his patient’s data and materials.

The researchers in Rotterdam established that it was not a paramyxovirus. The RNA sequences revealed it to be an unknown coronavirus, family of SARS and various cold-causing viruses. The virus would later be named Middle East Respiratory Syndrome coronavirus (MERS). Dr Zaki notified the Saudi Ministry of Health, in compliance with the international rules of the World Health Organization (WHO). He also posted the new virus on ProMED mail, the web and email service for outbreaks of infectious diseases. This was on Thursday, 20 September.

September 2012
On Saturday, 22 September, Ab Osterhaus, a renowned virologist of the Viroscience Laboratory of the Erasmus Medical Center, received a phone call from England. A man who had flown with a private jet from Qatar had been admitted to a London hospital. He had been isolated immediately and put on a ventilator. The man suffered from pneumonia and kidney failure and was in such bad shape that the British doctors believed he was probably going to die. They asked for the sequencing data of the new virus and probes – small parts of complementary RNA – to help them diagnose the virus.

This man also had MERS. He remained in intensive care for almost a year before he died. Within a week after the call from London, Bonn University in Germany, together with the Rotterdam Viroscience Lab, developed a specific test for the identification of the virus. There were just two patients at that time. Based on their experiences with SARS and Influenza, the virologists suspected that the virus had “jumped over” from an animal shortly before. But what animal species? They started to investigate.

Coronaviruses – “corona” is Latin for crown or halo – have a crown of spikes: proteins that can bind to receptors on human and animal cells. If such a viral protein binds to a receptor, the virus is able to invade and merge with the cell; the virus genome is then released and the cell starts to make new exemplars of the virus. Humans and animals that have once been infected with the virus have made antibodies against this specific surface protein (and others). If you know what the protein looks like, you can develop a test to identify these antibodies and also use this information to track down the receptor.

Autumn 2012
Bart Haagmans and Stalin Ray identified the human and animal protein DPP4 as the receptor. It is found on the outside of cells in the lungs, kidneys, intestines and on the cells of the immune system. This cellular protein is the key that the virus uses to enter the cell. Smaller animal species like mice, hamsters and ferrets proved not to be able to be infected with the MERS coronavirus. All animals share this receptor, but due to variation in the amino acid sequence there are differences that make it harder or impossible for the virus to bind.

Januari 2013
Another patient was diagnosed in London. A sixty-year old Pakistani man was admitted after a pilgrimage to Mecca and Medina in Saudi Arabia. His son and niece came down from Pakistan; they too fell ill. The son, a leukaemia patient who ran a greater risk, died within ten days. The niece recovered. The father died after weeks of intensive care. This case proved that MERS was also contagious between people. The virus would raise its head again in subsequent years, especially in hospitals in Saudi Arabia and the Gulf States, where a great many members of staff also died.
**Spring 2013**

Professor of Virology Marion Koopmans and Chantal Reusken of the RIVM National Institute for Public Health and the Environment in the Netherlands travelled to the Middle East to test large farm animals like cows, goats, sheep and dromedaries for antibodies. Dromedaries were found to be the only animals with antibodies against the MERS coronavirus. This was soon to be confirmed by other researchers. The antibodies were even found in the blood of dromedaries from the 1980s.

**April 2014**

In response to an outbreak of MERS in Qatar, the WHO asked Koopmans to investigate. Two people at a small farm were ill, and a number of dromedaries had some kind of cold. The patients and the camels were found to have the same virus. The question was: who infected whom? Koopmans focused on how the virus spread. In Qatar she examined different groups of animals: at an abattoir, in the market and at the dromedary races. Koopmans: “This region was booming and still is, thanks to the riches from oil and gas. There is enormous wealth and activity that draws a lot of people and this in turn creates a demand for food. Hence the huge market for dromedary meat. The animals are bred in great numbers. Qatar has one central abattoir. The country also imports a lot of animals from the Horn of Africa and Australia, and these all come together in the same market, where they are kept for months on end before they go to the abattoir. This truly is a ‘pressure cooker’ for the spreading of a virus.”

**May 2014**

Half of the dromedaries in the abattoir turned out to test positive for MERS. Koopmans wanted to push back the spread of the virus by, for instance, vaccinating camels from Australia – which were not infected – or by transporting them directly from the ship to the abattoir. “Transmission between people in the hospital can be managed by vaccinating hospital staff and good hygiene”, Koopmans explained, “but that will still leave the occasional animal-human infection. We want to block this path.”

**November 2014**

Ab Osterhaus and Bart Haagmans tested vaccines on dromedaries in Barcelona. In order to induce antibody production, they used the MERS protein that binds to the receptor. These antibodies then block the binding of the virus to the receptor; they stick to the virus so that it can no longer bind and infect the cells. Haagmans: “You can induce such a block beforehand with a vaccine even without injecting the entire virus. The binding protein may suffice.”

**December 2014**

It worked. The vaccinated dromedaries did not become ill, whereas the unvaccinated animals did. However, this was no guarantee for success. MERS did cause heavy sniffles in the unvaccinated dromedaries, but that was it. Chances were that the Saudis, for this reason, would have little interest in a vaccination programme. Osterhaus: “They also had an entirely different problem: camelpox. We put the MERS protein in a ‘vector’, a cripple pox virus that can no longer do any damage. This MERS vaccine, therefore, also protected these animals against camelpox. That would kill two birds with one stone and would probably make it quite interesting to the Saudis, we thought.”

**April 2016**

What about MERS today? In April 2016, the WHO counter for MERS worldwide reached 1,698 patients since 2012, including 609 fatalities. 

**“There is an entirely different problem: camelpox.”**
Immunologist André Boonstra focuses primarily on hepatitis B viruses. “The hepatitis B virus has a high replication speed over a long period”, he explains. “In other words, there is a continual development of numerous additional virus particles. Ninety per cent of adults nevertheless get rid of the virus by themselves. In ten per cent of cases, however, the virus triggers a chronic infection. In children, it is the other way around: the great majority of hepatitis infections in children become chronic.”

Booster for an exhausted immune system

Some viruses activate the immune system to such an extent that it wears itself out. The result is a chronic infection for which patients will have to take antiviral medication for the rest of their lives. There might be another way: give the immune system a shove in the right direction so that it can clean away the virus under its own steam.

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A look into immunologist André Boonstra’s lab.

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Such a chronic situation is dangerous. Because the virus divides itself so often it induces a lot of antigens, which the immune system sees as a threat. In response to this enormous amount of antigens, the immune system produces a great many virus-specific immune cells, mainly T-cells. Such a strong immune reaction may damage the liver, which is where the hepatitis virus settles.

To prevent serious damage, the body itself pulls the emergency brakes. If the immune system is exposed to a huge amount of antigens, the T-cells become exhausted and lose the ability to divide. This prevents further damage to the liver, but it also causes the virus to never be completely eradicated.

“What we have tried is to reactivate the exhausted immune system”, Boonstra explains. “This can be done in two ways. The first is to use exhaustion blockers. An exhausted T-cell is lined on the outside with so-called exhaustion markers, transfer substances that prevent overstimulation of the immune system by reducing the division of T-cells. By blocking these markers with antibodies, you can relieve the exhaustion and reinvigorate the T-cell response.”

The second method is to apply TLR-stimulation. TLR stands for Toll-Like Receptors. These are molecules on the outside of an immune cell that are able to recognise the structures of pathogens. They serve as the sensors of the immune system. By stimulating these sensors, you can reactivate the immune system.

Both strategies are potentially risky. There is a great risk of overstimulation of the immune system. Boonstra: “The existing therapies enable us to suppress the hepatitis virus quite effectively, so that few virus particles make it into the blood and liver. However, it is extremely difficult to remove all the virus particles from the body. You want to suppress the hepatitis virus in such a way that there are few virus particles in the blood, but more in the liver. Our idea is to treat people first until the virus is almost gone and then use this method of reactivation to give the immune system the last shove in the right direction. We will have to increase the dosage extremely slowly in the clinical trials.”

HIV infection, or AIDS, involves a similar problem. Virologist Rob Gruters is trying to stimulate the immune system to enable it to attack HIV. “HIV infects the cells of the immune system”, he explains. “This is itself quite nasty, but the virus makes things even worse by integrating its own DNA into the cell’s DNA. This way, it becomes part of the host’s DNA and can lurk in the dark. Moreover, the virus can change very rapidly. The result is a race in which the virus always stays one step ahead of the immune system. Eventually, the immune system gets exhausted and gives up.”
So in HIV cases there are two problems at play: the virus breaks down certain immune cells (CD4 T-cells) and eventually outsmarts the remaining immune cells. Problem number one can be tackled by the administration of antiviral drugs. The number of immune cells in the blood can be measured. Healthy people have 500 to 1,500 CD4 T-cells in a 1-microliter blood sample. In people with an untreated HIV infection, this number can fall to less than a hundred CD4 T-cells per microliter. Therapy is started when there are fewer than 350 CD4 T-cells remaining per microliter. Thanks to the antiviral medication, the number of immune cells rises again, but those cells will never be as effective as they were.

The second problem is more difficult to overcome. Gruters: “The immune system does recognise the HIV virus, but it looks at the wrong parts.”

Again, there is a risk of overstimulation of the immune system. An activated immune cell is ready to make all sorts of new products. This makes it an easy prey for the virus, which wants to make new virus particles. Inactive cells are far more difficult for the virus to successfully infect. If the immune system is activated but still does not manage to clear away all of the HIV, you might actually make it easier for the virus to spread.

Gruters compares the overstimulation of the immune system to an allergic reaction. “The immune system’s response to the stimulus that you apply is far too violent – it’s like cracking a nut with a sledgehammer. The sledgehammer does far more damage than required.”

Tackling hepatitis B and HIV is, therefore, quite a tricky challenge. On the one hand, you want to encourage the immune system to disarm the viruses, but, on the other hand, you do not want the immune system to be overstimulated. This calls for a subtle strategy.
In the 1990s, scientists needed hundreds of days, we were peeking through a keyhole, but now we can measure the activity of all the 20,000 human genes in one go. In the old days, we were peeking through a keyhole, but now we can open the door and have a view of the entire room.

Gene expression

We can view the room thanks to DNA sequencing: the sequential reading of all the genetic letters of the DNA molecule [see box]. The capacity of sequencing machines has continued to increase in recent years. “In the 1990s, scientists needed hundreds of machines and then it still took them ten years to map out the complete human genome”, says bio-information scientist Henk Jan van den Ham. “You can now sequence ten people’s genomes in three days with just one machine.”

The unravelling of dengue has been quite a feat: in collaboration with Indonesian doctors, Cox van de Weg and Eric van Gorp took blood samples of dengue infected patients and the expressions of all the 20,000 genes were measured. “You then know which pieces of the entire genome are on or off, but still have a long way to go, because you will also need to find out which of these genes are truly important. For some genes there is no question about it, but for thousands of others we do not know for sure”, Van den Ham explains.

Subtle differences

It is impossible to examine all genes one by one. Andeweg: “A pathogen’s antigen is firstly recognised by one cell in the immune system. But then all the cell reproduction starts, and you end up with a humongous population of immune cells. Thousands of genes activate themselves in these division processes, all adding to the enormous amount of data.”

The bulk of the data comes from cells that divide, but that is not what Andeweg and his colleagues are looking for. They are trying to detect subtle differences that determine whether someone has a good or bad immune response to a certain pathogen.

In order to discover those subtle differences, the researchers use co-expression network analysis. “Within the entire pattern of genes, there are groups of genes that show a specific type of behaviour”, Van den Ham clarifies. “So you can squeeze the original 20,000 measurements into small sets to create a network of genes. There are junctions in that network that might correspond to clinical variables.”

In the danger zone

Van den Ham uses an example to demonstrate this. “There will be groups of genes that are active but do not change their activity when you are infected. Take hearing. Genes that are key to hearing do not play a role in infection and so their activity remains low. For dengue, we have put the genes with the same behaviour in the same group. In the end we had over twenty of those groups, approximately half of which can be related to typical dengue symptoms, such as the amount of blood platelets in the blood.”

“If we know which genes are determinant for a certain illness, we can train the body to trigger the correct immune response”, Andeweg adds. For this reason, research into genes is also significant for the development of vaccines. In the case of dengue, such intervention is all the more important since the course of the illness is difficult to predict. “The majority of dengue infections cause no complaints, but fifteen to twenty per cent of patients do become ill. The first symptoms are somewhat similar to the flu. A few days later, it looks like the patient is recovering, but in a few per cent of them the infection then actually enters a critical stage, with leakage of the blood vessels causing haemorrhages all over the body.”

Dengue is spreading because the habitat of the mosquitoes that transmit the disease is continuing to expand. In other words, there are more and more people in the danger zone. That is why more knowledge of this unpredictable disease is more than welcome. ●

The dengue mosquito (Aedes aegypti) is the host of the dengue virus that causes dengue.
‘Innocent’ measles virus wrecks your immune system

Thanks to vaccination, measles has become a rare disease. But vaccination has become controversial, so even in this part of the world we have to deal with an outbreak of measles now and again. This emphasises the need for continued research into measles if we want to increase our understanding of the infection.

Rik de Swart, virologist at the Erasmus Medical Center Rotterdam, has been focusing on measles for many years. He met the Northern-Irish virologist Paul Duprex at a measles conference ten years ago. “Duprex delivered a presentation on a measles virus that makes GFP, a green fluorescent marker protein”, De Swart says. “The only problem was that he had no suitable model to test the fluorescent virus. We did, so ten years ago we decided to join forces. We now have a good model in which infected cells become visible – with a far greater knowledge of measles as a result.”

Measles was long considered a classic respiratory virus, a virus that infects the epithelial cells of the airways. But in 2000, a receptor for measles was discovered on immune cells. When De Swart and his team released the measles virus in the airways of monkeys, they saw that primarily the immune cells at the bottom of the lungs became infected. This was a strange place to find viruses, De Swart thought, knowing that the upper airways also contain cells that try to fight infection. “Dendritic cells – immune system cells – regularly stretch their new branches towards the upper airways in order to ‘feel’ if there are any invaders lurking about”, De Swart explains. “We believe that at such moment the measles virus seizes the opportunity and, hitchhiking on a dendritic cell, is taken along on this Trojan horse to the cells of the immune system.”

It is quite difficult for the virus to enter the gates as a hitchhiker, but once it is inside, it soon starts marauding, infecting various immune cells, including B-cells and different types of T-cells. The receptor to which the measles virus attaches can mainly be found on cells that have already come across parts of other intruders in the past. These cells are affected. At the same time, an effective immune response against the measles virus itself is triggered. The immune system clears away the affected immune memory cells. This in itself is, of course, good news: the patient gets better. But since the measles virus specifically attaches to immune memory cells and those cells are cleared away, patients actually lose their immunological memory.

Memory cells
De Swart: “We already knew that infection with measles weakens the immune system and also that this could last for weeks or months. We were also aware that although the number of white blood cells decreases during infection, it returns to normal values within a week. The thing we have discovered now, is that the numbers return, but the composition has changed. The cells that return are immune cells that fight the acute measles infection, but the number of memory cells that are effective against other infections has dropped.

Memory cells are immune cells that memorise what they have come across and can quickly jump into action at a second encounter with the agent of the disease, the pathogen. After infection with measles, a patient thus temporarily loses part of their immunity against other, primarily bacterial, infections. But temporarily here does not mean a few weeks or months, but more than two years. De Swart draws this conclusion from research in collaboration with American epidemiologists. They compared figures from databases from the United States, the United Kingdom and Denmark about mortality due to measles and other infectious diseases, both before and after the introduction of vaccination against measles.

More prone to illness
De Swart: “There is a clear correlation between the occurrence of measles and mortality due to other infectious diseases. This connection lasts up to approximately 28 months after a measles outbreak. This study stands out because it followed the same approach in the various databases and came up with same period for all the countries. After the introduction of vaccination against measles, the number of measles cases naturally decreased, but we can still see a higher mortality caused by other infectious diseases up to two years after an out-

This patient from Nigeria has a red rash all over her back - a well-known symptom of measles. At the moment that the rash occurs, the peak of the infection has already passed. That is why the virus is so contagious: other people are infected before the patients actually show clinical symptoms.
break. We therefore think that we have found a strong connection."

The researchers are now trying to find out if other databases reveal the same connection. GP databases, for instance, store anonymous data on patients' medical history. "We eventually want to examine the medical history of five hundred to one thousand children for a few years after they’ve had measles", De Swart continues, “and not focus on mortality, but for instance on the use of antibiotics and admissions to hospital. We would like to compare this group with children who have not had measles in order to see whether the first group really is more prone to illness in the two years after infection."  

Eradication  
Measles remain a significant health problem, as it is so unbelievably contagious. The virus has difficulties getting in, but is very effective in its way out. That is the trick. At the height of the infection (two to three days before people are actually showing any symptoms), the immune system is riddled with infected cells. At that stage, the virus also makes contact with the epithelial cells of the airways. This is how a huge number of virus particles end up in the upper airways. Patients can then very efficiently spread these virus particles throughout their environment by coughing. This compensates more than adequately for the difficult entry of the virus.

There is a vaccine, and human beings are the only natural host for the measles virus. Monkeys can be infected by measles, but the groups in which they live are generally too small for the virus to survive very long. Once infected, monkeys cannot be infected again, so in a small group of monkeys it does not take long before the virus cannot find another “fresh” host. The fact that people are its only host means that theoretically we could eradicate measles by vaccinating everyone against the disease. However, according to De Swart that is easier said than done.

“Vaccinations are certainly not undisputed”, he explains. "In a protected group it does not matter so much if a single member refuses vaccination. The vaccinated others will provide protection. But if the unprotected form a large enough group, the way to an outbreak is wide open – as we saw in 2013 in the Netherlands’ orthodox Protestant community."

Alternative vaccination  
Apart from the problem of non-acceptance, the vaccine itself is not always practical. It contains a live, weakened virus and must therefore be stored in a cool place. Especially in the tropics this is not always feasible. The consequence is that children are being vaccinated, but with a vaccine that is less effective. Moreover, the vaccine is administered with a syringe, which entails the risk of infection with HIV or hepatitis. De Swart and his team are seeking an alternative way of vaccinating that is better suited to the tropics.

“We are looking for a method that would allow people to inhale the vaccine through a nebulizer”, De Swart clarifies. "In our monkey model, we have already seen a good immune response if the vaccine ends up in the lungs. It is now in the form of a liquid solution, but it would be even better to store it in dehydrated form as the dry vaccine would remain stable for a very long time. That is what my American colleagues are currently looking into. We still have the problem, though, that you can never know for sure if someone has inhaled the full dosage. In other words, there is room for improvement of the measles vaccine, but it is still a long way coming. Until then, we want to use our research findings to reverse the image of measles as a mere ‘innocent’ childhood illness."
Hospital admission or not? Your genes decide

The respiratory syncytial virus (RSV) is an important cause of the common cold. The symptoms are usually limited to a runny nose in adults, but RSV infections can actually have very serious consequences in newborns. In fact, newborns may end up in the intensive care unit of the hospital and die if they go without treatment of an RVS infection. It would be great if there were a test that can predict which children will develop a serious form of the infection.

“RSV is one of the main viral infections in children”, paediatrician Pieter Fraaij explains. “Most children are infected for the first time in their first year. It starts with symptoms that are typical of the common cold, but in part of the children the symptoms worsen. One in a hundred children eventually ends up in hospital with respiratory complaints. Sometimes these are so serious that the child needs to be ventilated in intensive care.”

Blood samples
Which children run the risk of a serious form of infection is, however, difficult to predict. At the moment a child visits a GP or the hospital, it is hard to say whether it actually is RSV and whether the infection will intensify. That is why many children are referred to the hospital for observation - as a precaution. “This means that there are more children in hospital than necessary”, virologist Arno Andeweg adds. “We have searched the blood for biomarkers that can foretell whether a child will become seriously ill or not as soon as a doctor diagnoses RSV in a child.”

The research team collected blood samples from healthy children, children with a mild RSV infection and children with a serious RSV infection. The human genome boasts 20,000 genes, and the researchers went searching among them in order to find the genes that significantly differ in expression between children with a serious RSV infection and healthy or mildly infected children.

Each group included ten to twenty children. This means that the number of blood samples was far lower than the number of genes that were examined, and therefore classic statistics fell short. “That is why we used a method called machine learning”, says statistician Victor Jong. “Machine learning makes it possible to build a model step by step. You start off with the variable (in this case, a gene) that most strongly discriminates between a serious and a mild infection. You then add other variables, one by one, until the model’s predictive value levels off.”

Predictability
It works like this: suppose you are at 80 per cent predictability. When you add another variable, the predictability reaches 80.0001 per cent. “Would you then include this variable in your model? Probably not”, Jong says, “for it is of little use. Extra variables would make the model more complex, without contributing very much to its predictive force.”

The RSV model yielded 84 variables that considerably improved the predictability: 84 genes that play an important role in the development of a serious infection. The test’s accuracy is good:

between 96 and 97 per cent. So if the outcome of the test is that a child has a 10 per cent chance of developing a serious infection, this prediction is correct in 96 or 97 per cent of cases.

Understanding the outcomes
Whether the test also works well in practice has yet to be examined. Fraaij: “Measuring is easy. It usually is far more difficult to understand the outcomes.” Jong compares the test result to the weather forecast. “If there is a 90 per cent chance of rain, you take your raincoat. If a child has a 90 per cent chance of developing a serious form of RSV, it will have to stay in hospital. If there is a 40 per cent chance of rain, however, some people will take a raincoat and others will not. If a child has a 40 per cent chance of becoming seriously ill, the doctor will have to go by observations to decide on the next step.”

“Sometimes RSV is so serious that the patients need to be ventilated.”

“As a precaution, there are more children in hospital than necessary.”

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Vaccines do not work when they are left on the shelf

Virus infections can be needlessly problematic. For numerous viruses there are excellent vaccines, but these are not always available to everyone. Sometimes they are too expensive, and sometimes people do not want to be vaccinated because they don’t trust the system. This presents a problem: vaccines do not work when they are left on the shelf. Virgo coordinators Ab Osterhaus and Arno Andeweg explain.

Not all vaccines are available everywhere. What does this mean in terms of virus control?

Andeweg: “It certainly is an awful business that not everyone in the world has access to medication or vaccines. In the first place, this has to do with the unfair division of wealth. In the Western world we are quickly inclined to think that a virus no longer exists or is of interest, even if it is still causing major problems elsewhere. Hundreds of millions of people in the developing countries, for instance, fall victim to chronic viral hepatitis or the dengue virus. The availability of vaccines and proper medical care is extremely important – but (often very simple) hygiene measures are also vital.”

Osterhaus: “The distribution of vaccines and antiviral drugs in times of crisis is often closely related to the political situation. If a few countries buy up all the available vaccines when there is danger of a pandemic, there is nothing left for other countries. Pandemic vaccination should not only be available to the ‘happy few’, but to all who need it. We need to come up with solutions for this problem, for instance by procuring vaccines jointly within Europe. This is not something we scientists at Virgo are looking into, but sometimes we do see some very narrow escapes.”

Still, it is difficult to get everyone to accept vaccination, even in the wealthy Western countries. Take, for instance, the fuss over vaccination against HPV, the virus that causes cervical cancer.

Osterhaus: “I do indeed worry about the acceptance of vaccines. People are critical when it comes to science; they no longer take for granted everything their doctor says. On the one hand, there are religious reasons. There is little you can do about that. However, there are also highly educated people organising ‘measles parties’ because they think it would do their kids so much good. There is nothing healthy or good about it, but these parents do not know that. They have never experienced it themselves and are no longer aware of the consequences of the illness and the complications it might cause.” (See also ‘Innocent’ measles virus wrecks your immune system.)

Andeweg: “The fact that people do not trust the system unquestioningly is not a problem in itself. Everyone is an articulate citizen these days; the time when only the doctor knew what was good for you fortunately lies in the past. However, the increase of mistrust is worrisome. This scientific research aims to diminish the burden of illness, and this can only be achieved if the generated knowledge can actually be applied.”

How can we take away this mistrust?

Andeweg: “Sound information is important. The public’s lack of trust should stimulate us to share our knowledge more effectively. As scientists we have to be able to properly explain the pros and cons of a vaccination. Well-informed people can then make up their own minds, in their own interest and the interest of those around them.”

Osterhaus: “On the one hand, our field has achieved a great deal and is doing good work, but it is a pity that we do too little to promote the acceptance of the fruits of our labour: new vaccines. There are some horrible videos going around showing what supposedly happens after vaccination. No one takes a stance against these sceptics. It is up to science to counter their arguments. We should also appeal to social scientists and social media; they can help us learn more about the psychology behind acceptance and the best way to communicate.”

How does this complex problem affect your motivation for this research?

Andeweg: “Every researcher knows that their job is a long-term project: fundamental research in view of application in practice. We are used to being in it for the long haul; wars, poverty, and politics will always play a role.”

Osterhaus: “Our research is a dire necessity. We continue to see more and more infections. It is up to us to contain them – it was thanks to our approach to SARS, for instance, that we put a check on a rising pandemic. If we would not do this type of research, we would all suffer the consequences.”
VIRGO aspires to gain a better understanding of the main acute and chronic viral diseases, such as influenza, hepatitis, and AIDS. To this end, the consortium studies the mutual effects between the viruses that cause these diseases and their host on both a molecular and a physiological level.