Covid-19: unexpected mutations in SARS-CoV-2 variants continue to surprise virologists

Was the end of the Covid-19 pandemic announced too quickly? Experts are still trying to discover the conditions for the emergence of future variants and the drivers of the virus' evolution.

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Was the end of the SARS-CoV-2 pandemic declared too soon? While health policies have changed considerably and the much-discussed health pass seems to have been forgotten, virologists around the world continue to unravel the mechanisms of the virus' evolution. And if the avenues of research are becoming more refined, the conditions of emergence of the worrying variants, the risk of which has not been ruled out, remain a mystery.

Ever since the virus appeared, specialists have been thinking about its genome, the mechanisms that allow it to be transmitted and replicated in the human body, and its evolutionary system by comparing it to other viruses, particularly the flu virus. What is the system by which SARS-CoV-2 evolves and what is its fate in the human population, which was free of it less than three years ago?

They initially relied on the sequencing of circulating viral genomes, made possible by unprecedented genomic surveillance. The reference model is the seasonal influenza virus, which evolves according to a mechanism known as 'antigenic shift'. In a virgin, non-immunised population, the virus initially encounters no obstacles to its spread, but following vaccination or infection, collective immunity slows it down and the virus gradually evolves.

The limitations of the flu model

This is at least what happens with the seasonal influenza virus and drives its evolution in the human population. As the proportion of people who are immune increases, the dominant variant against which immunity is initially directed spreads less rapidly, gradually giving advantage to viral variants that are less susceptible to it due to slight changes in regions of the virus' surface protein as a result of mutations. The virus can thus persist in the population, and specialists rely on knowledge of this phenomenon to try to predict the next dominant influenza variant.

During the early months of the SARS-CoV-2 pandemic, genomic surveillance detected a similar phenomenon and virologists began to monitor recurrent mutations in the spike protein, such as the mutation at position 484, which seemed to herald the virus' adaptation to population immunity. Until another phenomenon interfered with this one and foiled their predictions, the sudden emergence of highly mutated variants not derived from the dominant variants.

"We realised very quickly that SARS-CoV-2 was not a virus like any other and that it would be difficult to combat," admits microbiologist Ravi Gupta

With the Alpha variant detected in the UK in December 2020, we saw the mutation at position 484 of the spike protein through genomic surveillance, and we expected a variant with this mutation to emerge epidemically," recalls bioinformatician Derek Smith, from the University of Cambridge, England. *But in the meantime, the Delta variant appeared, independently of the evolution of the Alpha variant. And then, with Delta, we had also seen many of the mutations that had already been seen in the Alpha variant and in the Wuhan strain, and again we expected the variants that carried them to take hold. But then we had Omicron with its 50 mutations and that really surprised us. We don't see that with the flu virus,"* he admits.

"Random phenomenon".

"In parallel to this mechanism of gradual evolution that we know about with the flu virus, we have these black boxes of worrying variants that we don't know how they arrive and that put all our expectations to shame. We are faced with a random phenomenon that is difficult to predict. As a result, we don't know how the next variant will emerge," adds Etienne Simon-Lorière, from the Pasteur Institute. "*Very quickly, we realised that SARS-CoV-2 was not a virus like the others, and that it would be difficult to combat,"* admits microbiologist Ravi Gupta, from Cambridge University. *"Unlike other viruses, it is able to infect a wide range of cells within the body, including nerve cells, heart cells, lung cells, intestinal and respiratory cells. It is also capable of infecting a wide spectrum of mammals, including, in addition to humans, deer, rodents and cats, which favours its circulation and evolution,"* adds the specialist.

Virologists were therefore faced with an enigma, and a new hypothesis quickly emerged to explain the emergence of these variants, independently of those circulating in the human population. It is possible that the virus has evolved on the fringes, in a niche that escapes genomic surveillance, within the human species itself, in immunocompromised people who would provide the virus with an ecosystem that would allow it to evolve at an accelerated rate.

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"In mid-2020, we realised that some people were persistently infected with SARS-CoV-2 and were shedding virus for long periods of time. Some people, who were immunocompromised due to cancer or other conditions, could be infected for several months," says Ravi Gupta. *"In these people, mutations appear gradually and this has also been observed in people treated with antibodies. This suggests that the virus can evolve within a single person leading to accumulation of*

mutations. We don't see such accumulation with genomic surveillance and it is very unlikely that such variants could have accumulated this pattern of mutations by circulating from one host to another," he says.

"Exhaust mutations

When the virus [SARS-CoV-2] *multiplies for a very long time in an immunocompromised person, whether due to chemotherapy or HIV infection, the variants that emerge from the evolution of the virus are no longer selected according to their ability to be transmitted between different people, but according to their ability to survive in that person's body,"* bioinformatician Darren Martin, from the University of Cape Town, South Africa, told us in late 2021. *"A cycle of virus escape and antibody neutralisation then begins. The virus is targeted by the antibodies and may acquire a mutation that allows it to escape. In response to this mutation, new antibodies are produced, but the virus will escape again and so on. If the infection lasts several months, the mutations accumulate,"* he said.

A guerrilla war, so to speak, of the virus against the immune system of immunocompromised people. Several studies have revealed the progressive appearance of recurrent mutations during this prolonged infection, some of which, located in the spike protein, are also found in the Alpha, Gamma, Beta and Omicron variants. This is the case for mutations in positions 484, 501 and 614, located more precisely in the receptor binding site, which is particularly exposed to selection pressure and therefore subject to mutations.

A British study also shows that SARS-CoV-2 infection can last up to 505 days in an immunocompromised person

According to a study recently posted on the MedRxiv website comparing data from different studies on a total of 28 immunocompromised patients and 168 SARS-CoV-2 genomes, mutations in the viral genome affect three properties of the virus: affinity for the ACE2 receptor, which allows it to infect cells that carry it, escape from the immune system and assembly of the virus before release from infected cells. "*The escape mutations found in Omicron or the Alpha variant are the hallmark of an evolutionary process within a single host due to pressure from the immune system,"* says Ravi Gupta.

Indices in wastewater

A South African study published in the journal *Cell Host & Microbe* in February assessed the degree of escape of a viral variant that developed in a person with advanced HIV infection from antibodies induced by different circulating viral variants or by the vaccine. The SARS-CoV-2 infection lasted for more than six months and the viral variant partially escaped antibodies induced by the Pfizer-BioNTech vaccine and, more strongly, those induced by the Delta variant, although the infection occurred before its emergence.

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A British study, the results of which were presented on 22 April, also reveals that SARS-CoV-2 infection can last up to five hundred and five days in an immunocompromised person. The study, conducted jointly by researchers from King's College London and Guy's and St Thomas' NHS Foundation Trust, which includes five British hospitals, is based on the follow-up of nine immunocompromised patients following transplantation, cancer or HIV infection. On average, these patients were infected for 73 days and in five of them SARS-CoV-2 mutated. "*It is important to note that, despite this, none of these individuals developed new variants capable of spreading like the variants of concern,"* the authors state in a press release, emphasising that these results are not sufficient to explain the epidemiological advantage of the variants of concern.

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The hypothesis that the variants of concern incubated in immunocompromised patients is also supported by the emergence of the Beta variant in South Africa, which was first detected in October 2020 in Nelson Mandela Bay, a South African metropolis with a particularly high proportion of HIV-infected people not receiving appropriate treatment. Studies into the origin of the variant have revealed that its ancestors were isolated in this region of South Africa.

A study published in February in the journal *Nature* on the presence of SARS-CoV-2 in New York sewage also supports this hypothesis, suggesting that variants with these escape mutations are chronically produced in human populations. The study involves the fortnightly monitoring, since January 2021, of mutations in a restricted portion of the spike protein gene containing the ACE2 receptor binding domain.

Very early on, researchers saw the appearance of mutations that had never been seen before and considered the hypothesis of an animal host where the virus would evolve, such as rats drinking sewage. But as knowledge advanced, researchers were able to match these mutations with those observed in immunocompromised people. "*We know that these variants were present in New York City sewage at the very beginning of the pandemic, between March and May 2020,"* reports virologist John Dennehy of the City University of New York, who led the study. *"We looked for the virus in rats to no avail, and as studies accumulated on the evolution of the virus in immunocompromised patients, we found that the mutations we had identified were also appearing in these patients, and they are found in Omicron,"* he continues.

The community trail

The intense diversification of Omicron lineages since its emergence in November 2021, however, qualifies this hypothesis. In addition to the multiple sub-lineages between which BA.1 and BA.2 have diverged, new lineages of the variant have recently appeared in South Africa, BA.4 and BA.5. According to some virologists, such divergence cannot be explained solely by the evolution of the virus during its circulation in the human population. It is necessary to imagine the existence of a reservoir from which these lineages were released. *"Omicron defies the hypothesis of evolution in a single infected person. That doesn't mean that the hypothesis is wrong, but it's not as simple as that,"* says Darren Martin. *"I think the theory of long-term infections is right. But the incubation took place in a community from which viruses are*

released into the general population. This could be a community of 10, 50 or 100 HIV-infected people not receiving treatment, as exists in rural areas here in South Africa or in other African countries. The Omicron virus circulates among these people, creating niches within which it diversifies, and the exchanges within this population also contribute to this diversification," he says. *"But this reservoir can also be an animal reservoir. There are long-term infections in animals,"* he concludes.

"A Chinese study reported similarities between mutations identified in SARS-CoV-2 genomes isolated from rats and Omicron, and suggested that rodent-human exchange of SARS-CoV-2 virus may have led to the emergence of Omicron," says Juan Ramirez of the Mount Sinai School of Medicine in New York.

Infrequent viral recombination

While they can mutate, the genomes of SARS-CoV-2 viruses can also recombine, a property common to all coronaviruses. When two variants of the virus infect the same cell, the enzyme that copies their genome can jump from one to the other to produce a chimeric genome. Several recombinations between Delta and Omicron and Omicron's BA.1 and BA.2 viruses have been detected sporadically through genomic surveillance. *"These recombinant genomes appear during periods of intense and simultaneous circulation of different viruses, as with the BA.1 and BA.2 variants. Ten to a hundred of them are detected each time, which can spread in a limited way in a given geographical area, such as in northern Europe. This has nothing to do with the scale of spread of the variants of concern,"* reassures Etienne Simon-Lorrière (Institut Pasteur). "*The fact that these recombinations can generate a virus with the advantages corresponding to the two original pieces of the genome is not worrying,"* he adds.

The researchers do not rule out the possibility that the virus could also evolve in certain animal species before being reintroduced into the human population. "*This is a risk that must be taken into account, as we saw at the very beginning of the epidemic with the mink farms in Denmark, where the virus was passed on to mink before being reintroduced to humans with a few mutations that were associated with escape properties,"* warns Etienne Simon-Lorière. *"It's more complicated, because the virus must first infect an animal and then be transmitted between animals, before reinfecting humans. It is easier to explain the emergence of variants of concern by infection in a human patient during which the virus will incubate for about a year and be reintroduced into the human population,"* warns John Dennehy.

"We need to increase sequencing, not only of the virus from the animal but also from the environment." Juan Ramirez, Mount Sinai School of Medicine, New York

However, a growing number of studies show that the virus circulates between humans and animals. It has been found in many species including white-tailed deer, mink, lions, cats, gorillas and several rodent species including rats and hamsters. "*We know that SARS-CoV-2 is a zoonosis and that there is therefore a circulation of the virus between humans and animals,"* Juan Ramirez points out. *"But most of the genomes sequenced so far are from humans, not animals. This limits our ability to visualise the circulation of the virus in animals, but we know that some variants circulating in the human population also circulate there, notably the Alpha, Beta, Delta, Gamma and Lambda variants. We need to increase the sequencing, not only of the virus from the animal but also from the environment,"* he insists.

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Of the more than 10 million sequences in the Gisaid bank, which allows the sharing of viral sequences isolated throughout the world, only a few thousand come from samples taken from animals, the rest being of human origin. An analysis conducted by Juan Ramirez's team on 3,595 of these sequences identified 128 virus lineages belonging to the different variants circulating in the human population in multiple animal species, including mink, white-tailed deer, cats and lions.

"Preventing emergence in the immunocompromised

In Ontario, Canada, a recent study of 300 white-tailed deer found the virus in 6% of them. *We have identified different lineages and the virus evolves as it is transmitted between fallow deer, but we are not able to determine whether the fallow deer is a reservoir for the virus,"* says one of the authors, biologist Jeff Bowman of the Ontario Ministry of Natural Resources and Forestry. In addition to the Danish study showing virus circulation between humans and mink, transmission of the virus from laboratory hamsters led to a human outbreak in Hong Kong in 2022.

While researchers continue to explore these hypotheses, uncertainties about the origin of the variants of concern limit public health response capabilities. "Until *we understand this mechanism, we can only be satisfied with detecting the emergence of such variants as early as possible, as was the case in South Africa with Omicron,"* acknowledges Etienne Simon-Lorrière. But the possibility of incubation in immunocompromised patients raises the question of monitoring the evolution of the virus in these people, who are already particularly exposed to severe forms of the infection. *If we want to combat the new variants, we must prevent their emergence in immunocompromised people by protecting them better with vaccines or drugs,"* warns Ravi Gupta. *But this is difficult, if not impossible, because the definition of immunocompromised people is so broad. It can range from tuberculosis to diabetes to transplant patients. Added to this is the fact that vaccine-induced immunity is limited and reinfections are possible. That's why SARS-CoV-2 is a big problem and we're not going to get rid of it just like that,"* he concludes.

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"Immunocompromised patients are a source of genetic diversity for the virus to adapt to evade neutralising antibodies or treatment, which means that at a population level the virus can resist immunity induced by vaccines or infection, as well as treatment," says John Dennehy. *"But the risk of stigmatising people and exposing them is significant.*

We saw this with the stigmatisation of Asian people after the emergence of SARS-CoV-2, and this dimension of the problem must be taken into account in prevention policies."

Catherine Mary