

# Vaccines beyond antibodies

*Spurred by pandemic research, are T-cell vaccines moving closer to reality?*

Anthony King\*

The new vaccines against SARS-CoV-2 elicited strong antibody responses in initial trials, which encouraged optimism amongst immunologists and public health experts who expected good efficacy. “With viral infections, it is almost unheard of to have a prophylactic vaccine that doesn’t work ultimately by generating neutralising antibody responses”, explained immunologist Kingston Mills at Trinity College Dublin in Ireland. However, the antibody response is not the whole story. “Efforts to explain how immunity is working against viruses to the general public has forced everyone to try to make things so simple that now what is left is a ridiculous oversimplified picture of the vertebrate immune system”, commented Antonio Bertoletti, infectious disease scientist at Duke-National University of Singapore. In fact, there is increasing research focus on the role of T cells in mediating the cellular response to infections and how to stimulate these cells through vaccines.

Antibodies work by recognising and attaching to surface structures of a virus or bacterium, which prevents the pathogen from infecting its target cells and mark it for destruction by other immune cells. However, pathogens can escape the antibody response via mutations that decrease the efficiency of antibodies from infection or vaccination. “You will still potentially get infected if you’re vaccinated, because the antibody response is not as strong as it was”, explained immunologist Luke O’Neill at Trinity College Dublin, Ireland. “But then the T cells will kick in and stop the virus when it is inside cells”. Simply put, antibodies tend to prevent infection, while T cells combat infection and illness. Specifically,

CD4 helper T cells primarily encourage B cells to generate antibodies whereas CD8 “killer” T cells eliminate cancerous and virally infected cells.

## Antibodies versus T cells

There were indeed glimpses of such an early T-cell response in COVID-19 vaccine trials. Evidence pointed to CD8 T cells protecting against severe COVID-19 just 10 days after the first jab of the Pfizer/BioNTech mRNA vaccine, when neutralising antibodies are barely detectable (Oberhardt *et al*, 2021). “In natural immunity, essentially the early T cell response is associated with less severe outcomes [from Covid-19]”, commented Alessandro Sette, an immunologist at the La Jolla Institute for Immunology in California, USA. As the infected cell is taken over by the virus to produce its own viral proteins, some of these peptides are transported to the surface of the infected cell and prominently displayed by the major histocompatibility complex (MHC) proteins. This triggers attention from T cells, setting in train an immune response to terminate the infected cell.

T cells may therefore become key for developing newer vaccines that protect against the increasing number of SARS variants that are able to dodge existing antibodies. Fortunately, T cells are not so susceptible to mutations on the surface of pathogens, such as the spike protein of coronaviruses, since they complex with MHC molecules from within the infected cell rather than large proteins or sugar moieties on the virus hull. “The T cell response is more diverse and not necessarily focused on that region of the virus that binds to cells”,

Sette explained. “It is more difficult for the virus to mutate all these different fragments”. Moreover, since individuals have different sets of virus-attuned T cells, the virus will find it much more challenging to evade T-cell immunity across a population. “To evade every T cell epitope would be ridiculously difficult for the virus to mutate away from”, commented Akiko Iwasaki, an immunologist at Yale University in the USA.

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But T cells have traditionally not been in the minds of vaccine developers. They are very hard to induce in humans—though easy in mice—they are much harder to study and measure than antibodies, and “modern” subunit and killed pathogen vaccines are poor at inducing CD8 T cells. Most vaccines were developed empirically, “on a look and see basis”, said Danny Altmann, an immunologist at Imperial College London, UK, “and they were assessed in terms of the antibody response they induced”. Admittedly, T cells received more attention in the wake of COVID-19 vaccines, but “usually figure 4 or 5” of academic papers, as Altmann put it. A macaque study also showed that transferring low levels of SARS-CoV-2 antibodies protected the monkeys, but only if sufficient levels of CD8 killer T cells were transferred (McMahan, *et al*, 2021). The same lesson

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DOI 10.15252/embr.202154073 | EMBO Reports (2021) 22: e54073 | Published online 18 October 2021

came from clinical observations. “People who develop an early and strong T cell response are most equipped to dampen and more rapidly eliminate the infection”, said Sette, “which is why strong CD4 and CD8 responses early are associated with less severe outcomes”.

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An analysis of vaccine trials found that the Moderna vaccine, followed by Novavax, then BioNTech/Pfizer and then Johnson & Johnson achieved the highest antibody titres (McDonald *et al*, 2021) whereas the highest T-cell response was induced by the Oxford/AstraZeneca vaccine. This might help to explain why the effectiveness of the Pfizer-BioNTech vaccine declined from 90 to 78% after 90 days, while the efficacy of the AstraZeneca vaccine fell less precipitously from 69 to 61%. “While the rate of protection from infection achieved by vaccination is reduced by time, the ability to control disease is more resilient”, noted Bertoletti, “and this might be explained by the presence of memory T cells that seem to persist for a long time [in vaccinated people]”.

### T-cell vaccines

Mills has focused on inducing T cells for a vaccine against whooping cough (pertussis), which has been resurging during the past decades. Some immunologists blame the switch from a whole-cell pertussis vaccine with many antigens to an acellular vaccine with a few antigens and alum as the adjuvant for selectively inducing antibodies, but weak T-cell responses. “We have very strong evidence that the current vaccine for pertussis induces very strong antibodies, but it doesn’t prevent nasal colonisation with the bacteria”, Mills commented. Experiments in mice have shown that if infected animals generate a strong T-cell response, “you get protection in the nasal cavity, which the current vaccine doesn’t”, Mills added. One consequence is that the bacteria—*Bordetella pertussis*—transmits widely amongst the vaccinated without causing much disease,

but it is prevalent enough to cause outbreaks where vaccination rates decline. Another issue with the current vaccine is that antibodies wane quickly, which is why children must receive up to five jabs.

A vaccine with a stronger T-cell response may also help in the fight against malaria. The reasoning requires a look at the parasite’s life stages. Once *Plasmodium* enters the body from a mosquito bite, the parasites migrate to the liver, where they infect and multiply inside cells. One approach is to induce enough antibodies to try stop all parasites reaching the liver. Another is to stimulate T cells to eliminate infected liver cells. “That’s where the CdAdOx1 [AstraZeneca] vaccine came from”, said Adrian Hill, an immunologist at Oxford University, UK, who developed this vaccine vector from chimp adenovirus as a chassis for malaria vaccines.

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The task of a malaria vaccine aimed at the liver stage of the parasite is to create a T-cell memory that will identify, locate and kill infected cells. Hill’s liver-stage vaccine initially achieved about 25% protection and increased it to 40% by giving a second malaria vaccine dose intravenously, which delivers more virus vector to the liver. Hill said, when they used the vector for a COVID-19 vaccine, they initially worried that it was designed for rousing T cells, not antibodies. Clinical trials, however, showed it inducing a reasonable antibody response and excellent T-cell response. “Viral vectors are just the best way of getting a CD8 T cell response, and importantly, better than RNA [vaccines]”, Hill claimed.

### Location, location, location

The quantities of T cells induced by a vaccine are not the only important measure. Location matters too. Donna Farber at Columbia University in New York, NY, USA, has studied memory T cells in mice infected with influenza, in particular a subset of cells in the lung that congregate around the airways. When these cells were isolated and placed into another mouse, they journeyed back to the lung and remained there. “Almost like they knew where they lived”,

Farber said. Further experiments underlined their importance in fighting off respiratory infections: “If you only had tissue-resident memory cells against flu—and you didn’t have antibodies or anything else—you were still really well protected against re-infection and against getting sick”, Farber explained.

“Tissue resident memory T cells are very effective at combating viral infections, because they are right at the site of viral entry”, explained Iwasaki at Yale. Not only does their location help; there are signs that they hang around for a long time. Farber looked at lung transplant patients and found tissue-resident T cells from organ donors 15 months after surgery. “We now know that there is a huge pool of tissue-resident T cells and what you are measuring in circulation is just the tip of the iceberg”, commented Wolfgang Leitner, head of innate immunology at the US National Institute of Allergy and Infectious Diseases (NIAID).

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Indeed, what is needed for more effective pertussis vaccine, according to Mills, is to induce T cells at the point of entry. This would not only stop severe illness but also clamp down on circulation of the bacteria. “Tissue-resident T cells are crucial for protection in the nasal cavity, and to a certain extent in the lungs”, said Mills. These cells can encourage a type 1 CD4 response that generates interferon gamma to interfere with viral replication, as well as interleukin-17 that revs up inflammation. In addition, the Th1 response calls in macrophages and cytotoxic T cells to stop bacterial and fungal infections in the nose. Another subset of CD4 helper T cells that seem important for protecting against pertussis are Th17s that recruit neutrophils to clear the bacteria from the nasal tissue.

Mills noted that the current pertussis vaccine fails to generate an adequate Th1 and Th17 response and does not promote tissue-resident memory cells (Chasaide & Mills, 2020). Alternatives are in the works: a live but weakened pertussis vaccine, a

vaccine based on the entire outer membrane of the bacterium and an acellular vaccine with a novel adjuvant to stimulate a stronger T-cell response in the respiratory tract, especially when delivered through the nose.

The same problem plagues the standard flu vaccine, which “is very bad at inducing cytotoxic T cells, because it is a killed vaccine”, according to Leitner. “It is a good inducer of antibodies, and we know antibodies are a correlate of protection of flu vaccines”. The vaccines protect against seasonal variants via antibodies, but this protection is relatively short lived. T-cell vaccines would do better through time, but developing this kind of vaccine has been challenging. “Lots of efforts have been made, by lots of people, to generate a T-cell vaccine, against flu in particular, but it hasn’t translated into a human vaccine that works based solely on T cells”, Mills said. CD4 T helper cells and antibodies are predominantly directed against viral hemagglutinin, which quickly mutates to escape antibodies. CD8 cytotoxic T cells mainly target flu nuclear protein which is generated inside infected cells, and presented as peptides complexed with MHC proteins. It is more conserved than hemagglutinin. Vaccinologists thus strived to develop a vaccine that would muster a battalion of these CD8 T-cell memory, to establish protection against new seasonal flu strains. “Unfortunately, all efforts to generate human vaccines based on nuclear protein have failed”, Mills noted.

### New strategies

Vaccine scientists have begun to explore new strategies. “To generate T-cell mediated protection, it might be a good idea to vaccinate at the site of infection”, Farber suggested. She compared injected and intranasal flu vaccines and found that tissue-resident T cells were only generated by the intranasal vaccine. More evidence of the importance of the administration route came from primate studies showing that intravenous administration of the BCG vaccine better protected macaques against *Mycobacterium tuberculosis* than intradermal or aerosol delivery: far more antigen-responsive CD4 and CD8 T cells were found in the blood, spleen and lung (Darrah *et al*, 2020). “There’s a lot of potential in terms of getting tissue resident memory cells from vaccines”, Farber said, “but it will require vaccines to be a little bit different”.

“Resident memory T cells are very effective at combating viral infections, because they are right at the site of viral entry”, Iwasaki said. She has developed a “prime and pull” strategy that seeks to establish T cells at mucosal sites. First her team vaccinated mice with a weakened strain of herpes simplex virus-2 (HSV-2), which is the prime step, and then topically applied chemokines to the genital tract to recruit CD8 T cells: the pull step (Shin & Iwasaki, 2012; Gopinath *et al*, 2020). The T cells, even without any viral antigens, tend to remain there. “We demonstrated that this prime and pull strategy can be a very effective prophylactic strategy for genital herpes infection”, Iwasaki commented, “and also demonstrated that recurrent genital herpes can be prevented or significantly reduced by this strategy”.

She has yet to do a human trial, but said the initial prime step could be carried out with any other vaccine platform, including viral vectors or mRNA. “As long as [the vaccine] induces T cells, all we have to do is get them in the right place”, Iwasaki explained. She and clinical collaborators are testing the concept amongst women with a precancerous cervical lesion: the women will receive the human papillomavirus (HPV) vaccine Gardasil (prime) followed by Imiquimod (pull), a trigger for Toll-like receptor 7 that has been approved for treating warts caused by papillomavirus topically. Iwasaki views her “prime and pull” strategy as suitable for respiratory pathogens too and is carrying out a study for COVID-19.

Arguably the trickiest pathogen to vaccinate against is HIV as the virus mutates rapidly to escape from antibodies. In addition, HIV incorporates itself into the chromosome of infected cells and can hide away, so antibodies must stop almost all viruses getting into cells. For two decades, researchers have tried to induce a strong CD8 T-cell response to eradicate all HIV-infected cells in monkeys. “Classical T cells have not been shown to respond effectively enough, or fast enough to contain HIV”, explained Dennis Burton, an immunologist at the Scripps Research Institute in the USA. In late August 2021, the NIH announced that a HIV vaccine candidate had shown insufficient protection against HIV infection in a study of 2,637 women in sub-Saharan Africa. The study (the Imbokodo trial) relied on four shots, two based on the adenovirus vector used by Johnson & Johnson for its

COVID-19 vaccine to trigger production of T cells. For immunologists, mRNA now shows more promise because dozens of antigens from multiple HIV variants could be included.

Yet, some still see glimmers of hope in T-cell vaccines. Louis Picker’s lab at Oregon Health Sciences University in the USA has used a weakened macaque cytomegalovirus expressing simian immune virus (SIV) proteins, which led proteins to be displayed to T cells with slightly different MHC rules. This vaccine protected 50–60% of monkeys then challenged with a highly virulent SIV challenge and led to the clearance of SIV, a relative of HIV (Hansen *et al*, 2019). “To me, this is the most interesting possibility. To combine broadly neutralising antibodies and T cells”, said Burton.

### An ancient foe

A more ancient foe is *M. tuberculosis*, which infects macrophages in the lungs and then usually goes quiet, breaking out to cause full-blown tuberculosis when the patient’s immune system wanes. CD4 T cells of the Th1 flavour are necessary for the control of TB, but vaccines to boost these cells failed. “Newer studies have shown that increasing Th1 responses do not necessarily lead to increased protection”, explained Rasmus Mortensen, vaccine researcher at the Statens Serum Institut in Copenhagen, Denmark. The focus has shifted to other cells, such as Th17 cells, B cells and CD8 T cells.

Mortensen believes that new vaccines should “stimulate arms of the immune system that the infection itself does not”, including Th17 cells. It still remains challenging, since TB becomes walled off in the lungs in granulomas, where they are difficult for T cells to access. Leitner agrees that we need to think beyond just antibody titres and CD4 levels. “Why is [*M. tuberculosis*] able to establish these very long lasting infections, and be the number one infectious disease killer in the world, if one immune parameter was going to decide its faith”, he said, referring to CD4 cells. Still, a protein subunit vaccine and adjuvant (GSK’s M72/AS01e) took many in the TB field by surprise when it showed good efficacy in an African phase II trial. Mortensen foresees that new adjuvants that stimulate other cells such as cytotoxic T cells or Th17 cells might assist in achieving a better TB vaccine. “We might be able to leverage all knowledge gained from the

development of SARS-CoV-2 vaccines”, he suggested.

### Increasing awareness

“To add more targets for T cell recognition to [Covid-19] vaccines is something that is worth considering and is being pursued by several different companies”, Sette said. One challenge is that T-cell stimulation and measurement tend to be quite variable between labs, and the community has not settled on a single test. “Anybody can run an antibody test”, Leitner commented. “With T cells, it is an artform”. There are two popular ways to measure T cells—enzyme-linked immunosorbent spot (ELISpot) assay, or flow cytometry. “There has been an emphasis on neutralising antibodies, and that is what everyone has measured for decades. Rightfully so”, Sette added. “But there is more awareness in the community now that you need also to measure T cells to have a more complete picture”.

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More evidence for the crucial role of T cells comes again from SARS-CoV-2. A recent study showed that people previously infected with endemic coronaviruses are equipped with CD4 T cells that cross-react with SARS-CoV-2, which might contribute to the “unexpectedly rapid induction of immunity”

following COVID-19 vaccination (Loyal *et al*, 2021). Another study indicated that recent infection with a “common cold” coronavirus resulted in less severe COVID-19 and fewer deaths (Sagar *et al*, 2020) with the authors speculating that localised immunity based on T cells may help to prevent lung injury (<https://www.the-scientist.com/news-opinion/common-cold-coronaviruses-tied-to-less-severe-covid-19-cases-68146>). And a preprint pinpointed T cells targeting a non-structural conserved part of the virus and suggested that these originated from previous coronavirus infections and contributed to the rapid clearance of SARS-CoV-2 in some people (preprint: Swadling *et al*, 2021).

Newer vaccines eliciting T cells against antigens different than the spike protein may therefore be more effective in protecting from severe disease and not infection, Bertoletti noted, and “they will likely also be less affected by new viral variants”. And if so, this could help to inspire other vaccine developments to put more focus on the T-cell response.

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