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Special Article

The past and future of vaccines – Reflections for discussion

Vacinas passado e futuro – Reflexões para discussão

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ABSTRACT

In this opinion article, I provide a brief history of vaccine development, commenting on the classic ways of producing vaccines using the infectious agent itself. I address viral vaccines, discussing their benefits and challenges and the issue of viral serotypes, as well as bacterial vaccines and their relative success. I present our studies on rheumatic heart disease and the development of a vaccine against streptococcal infection. I also discuss vaccine platforms, highlighting the success achieved with non-replicating viral vector-based vaccines and, especially, with messenger RNA (mRNA) vaccines. mRNA vaccines only became possible after the advances provided by the replacement of nucleotides that reduced the action of the innate immune system. Will all vaccines be made from mRNA in the future? Then, I address the issue of vaccine administration routes, whether subcutaneously, intradermally, intramuscularly, or intranasal. I present data from my laboratory on the development of an intranasal vaccine that induced a protective mucosal response, preventing infection and, consequently, the transmission of SARS-CoV-2. I discuss which future vaccines could be developed beyond acute infectious diseases. Finally, I discuss the advantages of developing safe, effective, multiple-use vaccines and how to make them accessible worldwide by promoting health equity.

Keywords: Vaccine, viral vaccines, infectious agent, bacterial vaccines, mRNA vaccines.

RESUMO

Neste artigo de opinião, apresento uma breve história do desenvolvimento de vacinas, comentando sobre as formas clássicas de produção de vacinas utilizando o próprio agente infeccioso. Em seguida, abordo as vacinas virais, discutindo seus benefícios e dificuldades e a questão dos sorotipos virais, bem como as vacinas bacterianas e seu relativo sucesso. Apresento nossos estudos sobre doença cardíaca reumática e o desenvolvimento de uma vacina contra infecções estreptocócicas. Também discuto plataformas vacinais, especialmente os sucessos alcançados com vacinas de vetores virais não replicantes e, acima de tudo, o grande êxito das vacinas de RNA mensageiro (mRNA). As vacinas de mRNA tornaram-se possíveis somente após os avanços obtidos com a substituição de nucleotídeos que reduziam a ação da imunidade inata. Serão todas as vacinas desenvolvidas a partir de mRNA no futuro? Em seguida, abordo a questão das vias de administração de vacinas, seja por via subcutânea, intradérmica, intramuscular ou nasal. Exponho dados do meu laboratório sobre o desenvolvimento de uma vacina de instilação nasal que induziu uma resposta de proteção da mucosa, prevenindo a infecção e, consequentemente, a transmissão do SARS-CoV-2. Posteriormente, discuto quais vacinas futuras poderiam ser desenvolvidas para além das doenças infecciosas agudas. Por fim, discuto as vantagens do desenvolvimento de vacinas seguras, eficazes e de uso múltiplo, bem como a forma de torná-las acessíveis à população mundial, promovendo a equidade em saúde.

Descritores: Vacina, vacinas virais, agente infeccioso, vacinas bacterianas, vacinas mRNA.

Vaccination began in the West with Jenner's administration of extracts of cowpox pustules (vaccinia) to humans. It was the end of the 18th century and despite the results of protection clearly observed against smallpox, much hesitation was shown in the use of this methodology, and it took the world 2 centuries to completely eradicate the disease with mass vaccination of the population. In

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this immunization, protection is provided through a cross-reaction between two closely related viruses: vaccinia, or cowpox, and human smallpox. While Jenner brought immunizations to the world, Pasteur, nearly a century later, laid the foundations for vaccines. Pasteur, working with animals, developed processes to attenuate the infectious agent or inactivate it, and several veterinary vaccines were developed for use. The biggest challenge, then, was to convey these principles to humans. Through multiple passages of the rabies virus in rabbits, he obtained the attenuated rabies virus, and then tested it in animal models. The opportunity to test on humans presented an opportunity when young Joseph Meister was attacked by a rabid dog and was in serious danger of death. The trial was an absolute success and was widely publicized, gaining the approval of politicians and the masses.

From that point on, much research has been done into new discoveries and many vaccines have been developed using these principles of cross-protection between similar infectious agents, or the use of an inactivated or attenuated infectious agent. We can say that for any infectious diseases for which an individual has contracted the disease once in their life and remains immunized, a vaccine can be developed. However, a scientific question arises when the disease does not directly induce protective immunity.

The most common reason for this evasion is the different serotypes of the infectious agents. Serotypes are small sequence variations in the target protein of the protective immune response, which mean that the antibodies no longer recognize this protein and, consequently, no more protection. Antibodies against this modified protein are needed, giving rise to a new serotype. A vaccine against this infectious agent should cover the various serotypes.

In this instance, finding a universal immunizer would be essential, but good results have not been achieved. For this reason, various vaccines have been developed covering the main serotypes or circulating serotypes, with the associated restrictions.

If we consider HPV, the first vaccine was made against the 4 serotypes known to be the most carcinogenic, and then a second vaccine with 9 different serotypes. This is an expensive vaccine to produce, with the inconvenience of adjustments to find a balance in the induction of a protective immune response compatible with the different serotypes.

The vaccine against influenza has to be updated every year; its composition is based on partial data on the incidence of the disease in the respective hemisphere in the previous year. Although the vaccine already has its immunogenic limitations because it is an inactivated virus, particularly in the population most in need, which is older people, its efficacy is often low due to the emergence of new strains not included in the vaccine.

The situation is even more serious with the dengue vaccine. We know that a second case of dengue tends to be more serious than the first one, due to what is known as ADE (antibody dependent enhancement), in which antibodies directed against the virus but which are not neutralizing, facilitate viral entry into the cells, increasing the viral load with important clinical consequences.

For this reason, the vaccine has to induce neutralizing antibodies in equivalent quantities, and a decline in titers over time can, instead of providing protection, induce severe disease.

This happened with a dengue vaccine produced by Sanofi Pasteur. After clinical trials, it proved to be effective in individuals who had already had the disease. Even so, after a few years it was observed that vaccinated children who contracted the disease had a more severe form of dengue than unvaccinated children, leading to a serious debate about the use of this vaccine in the general population.

As head of the Instituto Butantan, I coordinated the development of the attenuated tetravalent dengue vaccine. The viruses had been attenuated at the NIH, but the vaccine prototype had to be kept frozen and thawed immediately before use. Industrial production had very low yields and stability. From these viruses, we developed a system for mass production of the four monovalents under industrial conditions, improved their purification process and, above all, their stabilization, allowing them to be freeze-dried.

Instituto Butantan has developed a pharmaceutical product that can be used by the population from a prototype sent by the NIH. The phase II clinical trial was conducted at the Hospital das Clínicas Medical School at the Universidade de São Paulo (USP) in Brazil. It included more than 300 individuals, some had already had the disease and others had not. We then approved the phase III study, and trained 17 clinical study centers throughout Brazil, especially in regions endemic to the disease, to recruit 17,000 volunteers. The study was launched with great pomp

and circumstance in February 2016 in the presence of the President of the Republic, the Governor of the State of São Paulo and five Ministers of State, demonstrating how important this study is for the country. However, there has been a drop in the incidence of dengue in Brazil in subsequent years and, above all, there has been a decrease in the circulation of certain serotypes. As a result, it has not been possible to prove the efficacy of the vaccine for all serotypes. For the serotypes studied to date, protection is above 80%.

Despite these achievements, the development of a vaccine for the different viruses that the immune system is unable to eliminate is very difficult. The most important example is a vaccine against HIV. Despite enormous progress in our knowledge of the pathology of this disease and advances in the antiviral immune response, the characteristics of the virus elude the immune system and, despite multiple attempts with billions of dollars invested, there is still no good candidate for a vaccine. Many different antiviral therapies have been discovered, which is a great success.

However, researchers have observed that some people living with HIV for years have developed antibodies with a high neutralizing action. Several monoclonal antibodies with promising therapeutic action have been obtained from the B lymphocytes of these patients, especially if they are combined; however, they are still in clinical trials and have not been applied in clinical practice.

The epitopes recognized by these monoclonal antibodies, thus identified, may shed light on the development of an effective vaccine for the prevention of HIV, or at least for therapy to reduce viral load, drastically reducing transmission.

While vaccines for viruses are an undeniable success, antibacterial vaccines are more complex and often not as effective.

The classic BCG vaccine developed in France is widely available in Brazil, but it is not unanimously used worldwide. Difficult to mass-produce, it is now rarely available on the market. With the difficulty of a controlled phase 3 clinical trial, real-life observations of efficacy show that BCG does not prevent tuberculosis but slows it down, and cases of severe tuberculosis are rarely observed in vaccinated individuals.

Although this vaccine has been used in Brazil, the disease has resurfaced in recent years, even with constant surveillance. This resurgence is probably due to the greater susceptibility of patients living with HIV, who also produce bacilli and spread the disease.

Another classic vaccine, DTP is a great success worldwide. It has been used for decades, practically eliminating diphtheria in the countries where it is used, and greatly reducing tetanus, a significant cause of infant mortality in Brazil before the vaccine was introduced.

The pertussis component, caused by the bacterium *Pertussis*, has also had great success in preventing the disease in Brazil. In recent years, several countries, including France, have started using the acellular *Pertussis* vaccine. With new technologies using isolated proteins, this acellular vaccine avoids the adverse effects of administering the whole inactivated bacterium, but its effectiveness over the years has proved much lower, with a drop in protection over time. In the various countries that have adopted it, there has been a resurgence of the disease, especially in older people, where it can be very serious. In addition, adults can present with a mild form of the disease and carry the *Pertussis* bacterium with an enormous risk of transmission to newborns, when the disease is often fatal.

The pneumococcal vaccine is extremely important, especially for older people, but it is relatively effective and very difficult to produce. As the protective response is fundamentally against the sugars in bacterial glycoproteins, a large number of components need to be synthesized in order to obtain only partial protection.

My research group has been working for many years to understand the immunopathological mechanisms of an autoimmune disease triggered by a bacterial infection: rheumatic heart disease, triggered by pharyngitis caused by *Streptococcus pyogenes*. There are over a hundred serotypes of *Streptococcus pyogenes* due to variations in the amino acid sequence of the N-terminal region of the M protein, which is the main antigen of this bacterium. We describe how T-cells cross-recognize streptococcus M-protein and vimentin from the cytoskeleton of cardiac cells. As these lymphocytes migrate to the valves, they lose control of the regulatory cells and cause vegetative lesions, altering valve function.

We described the main susceptibility genes for the disease, in which the HLA-DR53 molecules stand out. From there, we mapped the humoral and cellular response against synthetic M protein peptides and identified a region with a T epitope, followed by another

with a B epitope, which are associated with protective responses.

We have thus described a synthetic peptide vaccine, which has been tested in various animal models and it has been possible to characterize a good cellular and humoral response. The serum of the immunized animals induces opsonization of the bacteria by macrophage cells, and they are protected against the challenge of a fatal inoculum with streptococcus. In addition, the vaccine does not induce any autoimmune manifestations in mice or minipigs, nor in mice genetically modified with the HLA DR genes, which give susceptibility to the disease.

This vaccine is due to enter a phase I clinical trial soon. If we get the expected results, we could prevent childhood angina, and above all the serious rheumatic heart disease that kills thousands of people, mainly in Africa and Southeast Asia.

If bacterial vaccines are limited both in their number and actual efficacy, anti-parasite vaccines are even more challenging. Parasites are complex organisms with multiple ways of evading the immune system. In addition to changing their form, they also alter the expression of their antigens in the multiple forms they acquire. There are some vaccines against Leishmaniasis with limited efficacy in humans, and recently some reasonable results in a vaccine against malaria. A great deal of scientific knowledge is still needed to understand how we can be successful in producing vaccines against parasites.

The vaccines using the active and inactive infectious agent that were possible to develop have already been developed. Now science has to go beyond nature to produce vaccines with ingenuity using the important part of the infectious agent, and this translates into identifying the targets of the immune response, whether through antibodies that neutralize the infectious agent, or the CD4 lymphocyte response for auxiliary effect and the CD8 response with cytotoxicity eliminating infected cells. The targets comprise the so-called antigen.

The vaccine needs a component that links the innate and adaptive immune responses, which will serve as a spark to ignite the fire of protective acquired immunity and its respective memory. For this, an adjuvant is used, which is an inflammatory chemical or the vector vehicle itself, which can be a non-replicating virus, a virus-like particle, or another type of nanoparticle that includes nucleic acids such as RNA or DNA inside, which alone activate innate immunity.

Some years ago, the international scientific community was aware of the possibility of a pandemic due to the densification of cities and the ease of transportation of people around the planet.

In February 2017, I participated in the inaugural symposium of CEPI (Coalition for Epidemic Preparedness Innovations) in which I spoke about the ZICA epidemic and its possible consequences in Brazil and around the world. The event was attended by the then French President, François Hollande, to show the importance given by France and Western countries to the possibility of a pandemic, and how to prepare for it. The meeting was a success and CEPI was given resources to invest in a major program so that the groups, through their platforms, could be more agile in developing drugs and vaccines for possible pandemics.

The most relevant platforms that were already underway and which have emerged with great force and efficacy during the COVID-19 pandemic are nonreplicating adenoviruses vectors and messenger RNA.

Adenovirus platforms have the antigen gene incorporated into the viral genome. These viruses are nonreplicating vectors because they can infect but do not multiply in human cells. This is the case with the chimpanzee adenovirus used by Oxford University, which gave rise to the vaccine marketed by Astra Zeneca. The other system used is human viruses such as the Ad26 used by Janssen or the Ad5 used by Cansino and both used by Sputnik. In these cases, one of the genes essential for viral multiplication in human cells is deleted from the virus so that it cannot multiply. For industrial production, this virus is inserted into the cell used for viral replication and once the viral mass has been produced and the virus is isolated, it no longer multiplies. When the vaccine is injected, the virus attaches to the cell, injects its nucleic acid content which will be translated into viral proteins and also into the antigen that will be secreted and induce the expected immune response. In vaccines against COVID, this platform has been used extensively, as the companies mentioned above have shown. These vaccines induce a good protective immune response and have been widely used around the world. The major problem experienced with using this platform on a large scale was the adverse effect of inducing thromboembolic thrombocytopenic purpura. This rare effect was observed mainly in young women, and raised much concern about the widespread use of this type of vaccine vector.

Undoubtedly, the greatest success that arose from this pandemic was the emergence of the messenger RNA platform. This represents the development of many years and many researchers, and humanity was fortunate that technology was at a level of advancement that allowed effective and safe vaccines to be developed in remarkable time. Treatments for both defective gene replacement and cancer and vaccines using messenger RNA have been sought for many years, yet some almost insurmountable difficulties existed, the most important of which is that RNA is extremely inflammatory. The signaling system of innate immunity through Toll receptors perceives RNA as if it were a virus and triggers an explosive response. Things have changed thanks to the efforts of Hungarian biochemist Katalin Karikó in collaboration with immunologist Drew Weissmann at the University of Pennsylvania. They showed that much of the toxicity was due to the RNA nucleotide uridine and that the use of pseudouridine, which maintained the same coding, suppressed recognition by innate immunity. The other fundamental discovery was how to encapsulate the mRNA molecule, which protected it from RNAses, allowed the nucleic acid to be fixed and incorporated into cells, allowing it to be expressed and exported extracellularly, triggering a powerful immune response. These lipid nanoparticles have various components, including cholesterol, phospholipid, and polyethylene glycol. Given the ease of synthesizing the mRNA *in vitro* and the improvement in encapsulation techniques, after the sequence of the spike protein was published, the vaccine had already been synthesized in good manufacturing practice in 66 days, all immunogenicity and toxicity tests had been conducted, and clinical trials had been started. Truly a milestone in world science. This technology will undoubtedly be improved in order to better control the length of time the RNA remains, the amount of antigen produced, and to control some adverse effects. Some of these effects are due specifically to the spike protein and should be further investigated. In 2018, in an article published before the pandemic, our group warned that PEG could trigger anaphylactic reactions when used in medicines, a fact that has been observed with these vaccines. Also, with RNA vaccines, the inconvenience is that they have to be stored at ultra-low temperatures, which poses many logistics challenges, especially in poorer countries.

In Brazil, these issues have been resolved to some extent, as the most remote populations, such as those living on the banks of the Amazon River, for example, do not have access to these vaccines.

There are other platforms being developed, many of them based on virus-like particles (VLP), in which the main antigen is included in a protein-only composition, which is uninfectious but resembles a virus and is in turn very immunogenic; other vaccines are composed of antigenic proteins that are formulated with potent adjuvants and induce a strong immune response.

These platforms had all been developed and when SARS-CoV-2 emerged at the end of 2019, and the spike protein sequence in February 2019, these different groups used the spike protein to develop their vaccines. The spike protein was known to be essential for the penetration of the virus into the cell and these findings had been described in studies conducted with the SARS-COV strain that appeared in China in 2002/2003.

In addition to vaccine production platforms, administration routes are also evolving. These rapidly developed vaccines are all intramuscular, but we know that the subcutaneous route is very important because it induces high and efficient responses. The intradermal route, using micro-needles, activates Langerhans cells. These are vaccines that, with a small amount of antigen, induce extremely high responses and, in terms of protecting mucous membranes, we need to think about other forms of immunization.

Since March 2020, our group has been working on a nasal instillation vaccine for COVID. At the very beginning of the pandemic, we recruited 250 individuals who had been infected with COVID, studied the antibody response in detail and saw that in fact a response against the RBD (Receptor Binding Domain) was sufficient. The spike did not need to be fully utilized, and this protein may be involved in the adverse reaction processes we have observed. We also studied using algorithms which were the best antigenic determinants for CD4 and CD8 cell response, synthesized 67 peptides from the 32 viral proteins and selected 14, 7 CD4 and 7 CD8, which cover the interaction with HLA molecules to be presented and induce a good T-cell response. This RBD and the peptides were placed in two types of nanoparticles after testing more than 50 formulations. The vaccine thus prepared was used through nasal instillation and induced a systemic response with a production of circulating neutralizing IgG and also T lymphocytes that also recognize the virus. Above all, however, we observed an IgA response in saliva

and bronchoalveolar lavage, which is what we were aiming for. A response of this type of immunoglobulin, which is responsible for protecting the mucous membranes. This type of vaccine, which is currently in the production phase of pilot batches for human trials, aims to produce an RBD vaccine that can be changed very easily, depending on which variant is circulating, and also to induce an IgA response that will prevent infection of the nasal mucosa, so that the disease will not spread, viral replication will be prevented, transmission among asymptomatic individuals will cease, and we think that we can therefore completely control the pandemic.

Science has achieved a new record in 11 months: after the appearance of a new disease, a vaccine was widely applied and the pandemic has been controlled, or at least the incidence and mortality of the disease has been reduced. Whether the pandemic will last is yet to be seen, as is the question of how long the protection acquired through vaccination will last. What has the medical community thinking hard is the low uptake of vaccinations in many places. The incessant work of antivaccine groups, preventing the population from getting vaccinated and presenting some adverse effects, which have in fact occurred, as if they were generalized to everyone. Different means of communication should be used to educate the population, not just the classic media such as television, radio, and newspapers, but digital media such as social networks in order to reach all strata of the population.

In the US, where RNA vaccines have been highly effective, only two thirds of the population has been vaccinated. More importantly, the unvaccinated population generally comes from the poorest and least educated backgrounds. However, antivaccine movements are important in the most educated population - this is a major problem for the advancement of vaccination worldwide and a joint effort across all academia would be very valuable.

It is worth noting that in the future with all these platforms, studies and advances in immunology; it may be possible to make vaccines against something that we are not currently considering, such as infectious diseases that invade the immune response in general, against cancer. Today we have the anti-HPV vaccine, in which the virus induces cancer, so it could be considered an anti-cancer vaccine.

In the future, we could make vaccines against specific cancer antigens, or immunotherapies against these antigens using the vaccine principle and checkpoint inhibitors together.

For allergies and asthma, we could also, in the future, have immunizations against allergens in order to induce IgG and not IgE, which causes the disease. For autoimmune diseases, we could have immunizations every time we find out which agent triggers them, as many of them are triggered through infectious agents, such as rheumatic fever, and perhaps for degenerative diseases, in many of which there is an immunological component in the degenerative mechanism.

This is a very broad field, and vaccines can be used to provide top-quality preventive medicine.

Finally, may I remind you of what vaccines bring us. A vaccine is the perfect medical act, because it prevents disease and suffering! It is easily applicable to the population and the cost is very low. It reduces infant mortality while increasing life expectancy. However, this information needs to be disseminated to the population. New technologies and new vaccines will come along and they will have to prove their benefits. Let us strive for a healthier world and greater equity, with greater access to health care to prevent the diseases that afflict humanity.

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