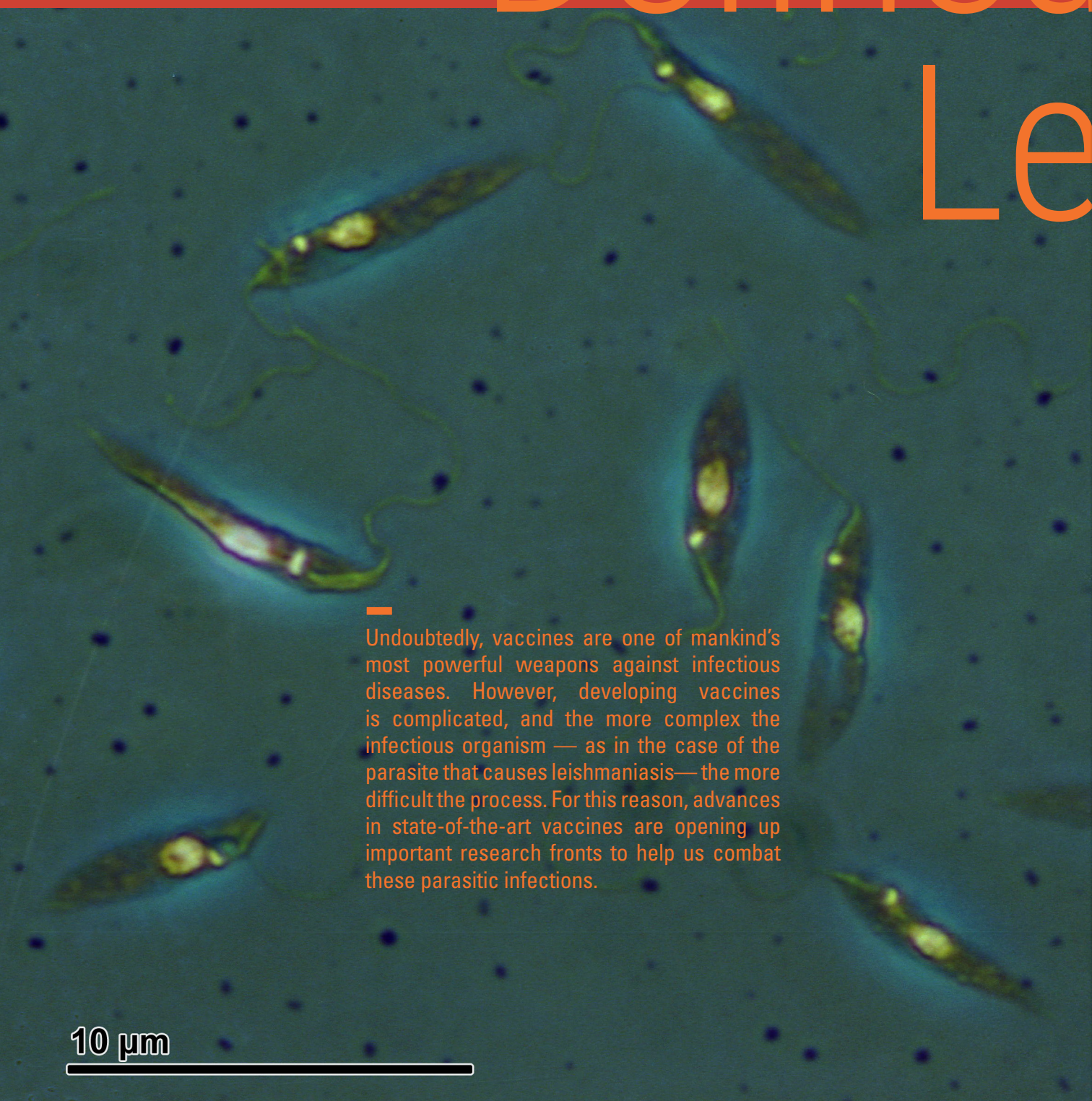
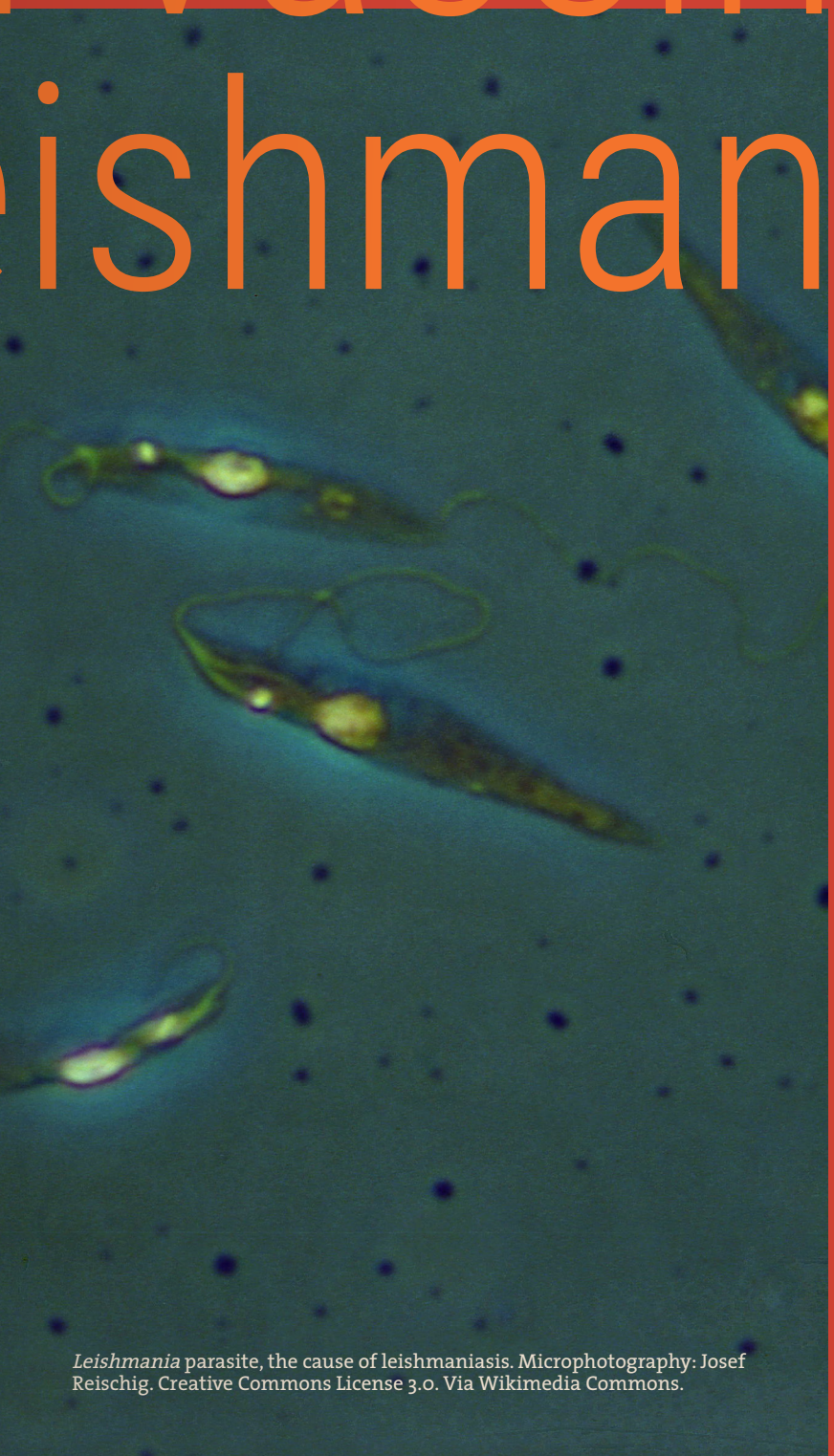
Developing a Defined Le



Undoubtedly, vaccines are one of mankind's most powerful weapons against infectious diseases. However, developing vaccines is complicated, and the more complex the infectious organism — as in the case of the parasite that causes leishmaniasis— the more difficult the process. For this reason, advances in state-of-the-art vaccines are opening up important research fronts to help us combat these parasitic infections.

a Chemically
l Vaccine for
eishmaniasis



Leishmania parasite, the cause of leishmaniasis. Microphotography: Josef Reischig. Creative Commons License 3.0. Via Wikimedia Commons.

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Vaccines are wonderful tools invented by mankind to fight infectious diseases with a surprisingly favorable cost-benefit balance. They act in a very interesting way: essentially, they trick the body into believing that it is being infected by some agent that can cause disease, what we call a pathogen. This activates the immune system so that it registers the characteristics of the invader, memorizes them and, thus, remembers the invader if it ever actually enters our body. In that case, we will be ready to react strongly and quickly to limit the multiplication of the infectious agent through proteins called *antibodies* or through a *cellular response*, as shown in **Figure 1**.

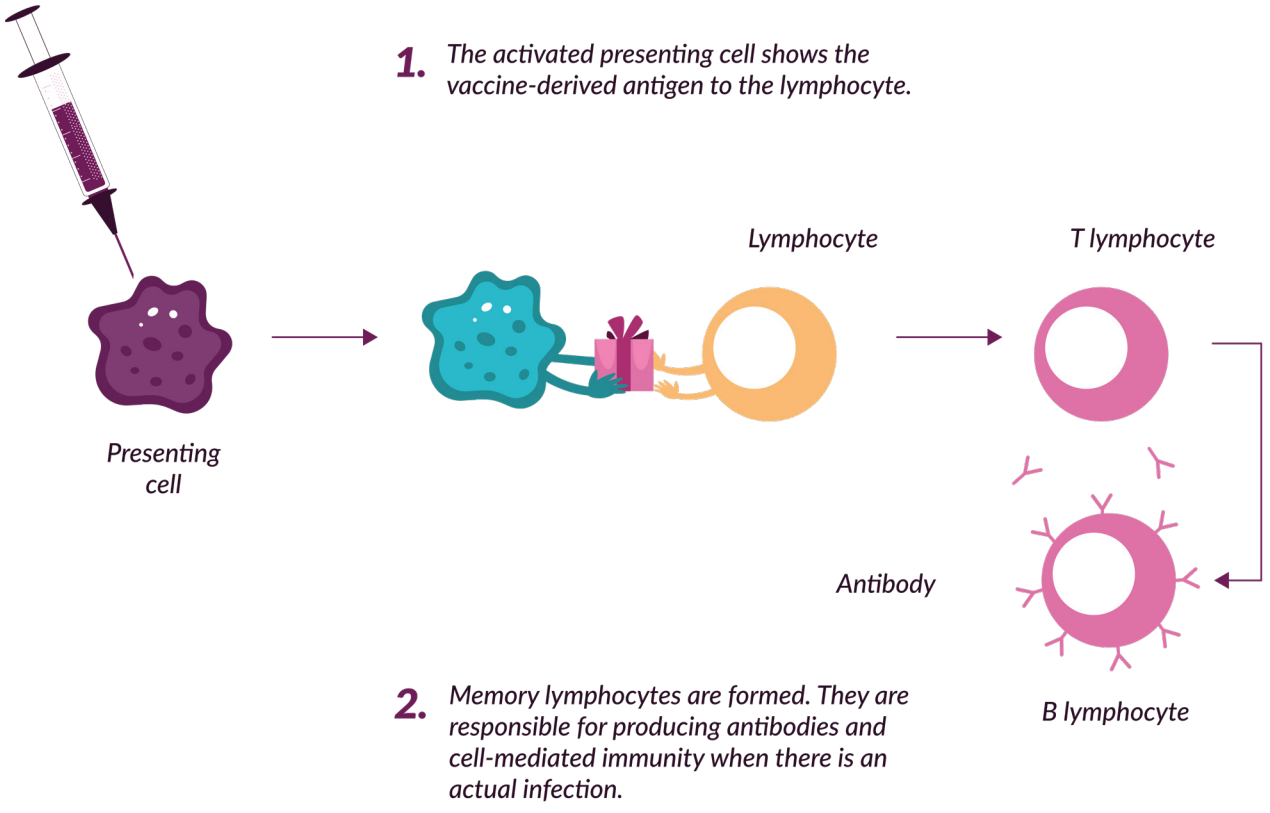

Figure 1. How a vaccine works. Infographics: David Bautista/ Carolina Gomes

Traditionally, vaccines use either complete pathogens that are attenuated —their ability to cause disease has been diminished— or inactivated pathogens. These pathogens have a set of antigens: subs- tances that, upon entering the organism, generate a specific reaction from the immune system. Thus, what a vaccine seeks is that the immune system recognizes one of them in a controlled manner, without causing all the symptoms of the disease.

However, this type of vaccination involves certain risks for some immunosuppressed people: Those who have their defenses at a very low level because of a previous condition or clinical therapy. Additionally, the large-scale manufacture of these vaccines is quite complicated. This is why vaccinology has evolved: The new vaccine generation tries to use only one antigen, a part of the pathogen that has the capacity to generate a relevant stimulus for the immune system and helps to recall the whole germ. These new vaccines are called *molecularly defined vaccines*. They are relatively safer and easier to produce on an industrial scale.

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Developing Vaccines: a Complex Task

Due to the situation the world is going through with the pandemic of the new coronavirus, society has been able to catch a glimpse of the complex process of vaccine development. At the moment, several vaccines for covid-19 are already being used to counteract the pandemic. They have gone through clinical phases to evaluate their efficacy, that is, the protection against the disease, and safety: the generation of as few adverse effects as possible. This stage was reached after a series of preclinical phases, that is, the stage before human trials. Preclinical phases are carried out in cell cultures in research laboratories and through experimentation with non-human animals.

Usually, the development of a vaccine takes about a decade, but in emergencies, this period should be cut to a minimum without sacrificing scientific rigor and ethics. Therefore, some people might ask why there are still no vaccines for many other infectious diseases if progress has been made so quickly in a vaccine for the virus that causes the pandemic.

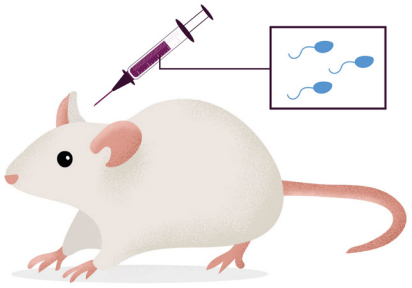
The answer can be summed up in one word: complexity. For example, the microorganisms that cause malaria or leishmaniasis are very large, from a genetic point of view, which means that they can express many more different molecules than a virus inside them. In addition, the life cycle of these parasites is intricate, and they have established a complicated relationship with humans throughout evolution.

For example, they can manipulate the immune system of the organism they infect at their convenience. All these phenomena make

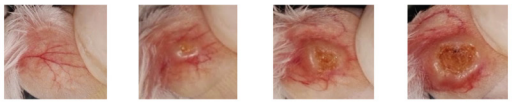
finding the antigen, the molecule that generates protective memory against these parasites, an arduous task.

In Universidad de Antioquia's Immunomodulation Group (GIM), we have been working for more than a decade in the search for a vaccine for one of these tropical diseases: leishmaniasis. Initially, we were able to establish an animal model: We simulated the human infectious process in mice.

This has allowed us to study some important aspects of the infection and search for prevention and treatment strategies against this disease **(Figure 2).**



1. *Leishmania parasites are inoculated into the mouse's ear.*



Week 1 *Week 3* *Week 5* *Week 7*

2. *Week by week, after the infection is inoculated, the development of the lesion is reviewed.*

Figure 2. Experimental model to study cutaneous leishmaniasis. Infographics: David Bautista/ Carolina Gomes

Leishmaniasis is a neglected tropical disease caused by a parasite transmitted by an insect bite. This disease is present in almost all of Colombia and is related to rurality, poverty and the armed conflict. Although the cutaneous variety, which is the common one in Colombia, is not fatal, it is painful, and there is a great stigma associated with it because it disfigures the skin and mucous membranes. There is no vaccine for this disease, and treatment is very deficient: costly, toxic and difficult to access, among other limitations.

After we had the model, we tested a basic, highly effective vaccine system. It consists of a combination of all the parasite's proteins —which are its antigens— with a substance that helps the immune system enhance the response to that antigen — adjuvant— called CpG. We also observed that a structure called a *liposome*, which can encapsulate antigens, increased the vaccine's efficacy and protected it from deterioration. This story was published in the first issue of the journal Experimenta in 2014. However, these vaccine formulations were not molecularly defined.

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Towards New Generation Vaccines

Our challenge in recent years has been the development of a molecularly defined nanovaccine, and thanks to research projects funded by the Ministry of Science, UdeA's Committee for the Development of Research (CODI) and Corporación Universitaria Remington, we have made significant progress towards meeting this goal.

To begin with, we must bear in mind that *Leishmania* parasites have about 8,000 proteins, so the search for a small group of defined antigens that are candidates for vaccination is like looking for a needle in a haystack. Although many of these proteins go unnoticed by the immune system when the pathogen invades us, others may either elicit a favorable response for the proper resolution of the disease or, conversely, elicit an unfavorable response that benefits the survival of the pathogen.

Our idea was to determine which proteins are detected by the immune system, which we call "immunoreactive", and which of these generate a favorable immune response that we can take advantage of to formulate a molecularly defined vaccine. For this meticulous search, we used a methodological approach called *immunoproteomic*, which explores the interaction of the host immune system and the set of proteins of the pathogen to establish which antigens of the microorganism induce a specific reaction in the mouse's immune system.

The parasite's proteins are separated in a gel according to their electrical charge and size with a technique called electrophoresis. Then, they are transferred to a piece of special paper. This set of proteins of interest, called the *proteome*, is brought into contact with the serum of animals that are immune to the infection either because they were cured of a first infection or they were vaccinated with our basic vaccine system. Antibodies produced in response to vaccination or infection

are present in the serum of the mice. After bringing proteins and sera into contact, the *immunoproteome* is revealed **(Figure 3)**. We can see in it which proteins are recognized by a specific type of antibody that is related to a protective immune response. These proteins become our potential candidates to be part of an effective vaccine.

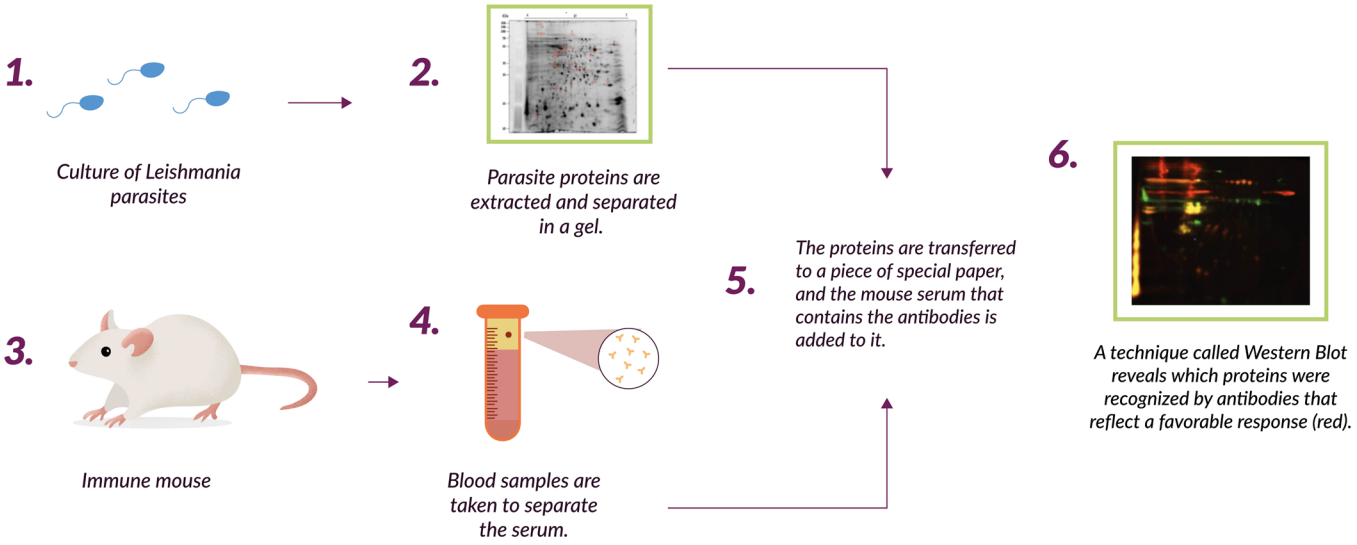

Figure 3. Immunoproteomics, a tool for finding the needle in the haystack. Infographics: David Bautista/ Carolina Gomes.

Testing Our Candidates as Recombinant Protein or DNA Vaccines

Once the proteins are identified by their full name —by finding out their sequence and their function in the parasite— they can be obtained/formulated in different ways. One of them is by generating a *recombinant protein*, which works as the vaccine antigen. On the other hand, DNA vaccines, also called genetic vaccines, can be used as well. These vaccines use a small piece of genetic material that temporarily instructs the cells of the vaccine recipient -in this case, mice- to produce the desired antigen. Thus, we decided to test both methodologies to select the most promising molecularly defined antigen from a small number of candidates.

In the experiments, we found that the "TR" protein was our best candidate, as it provided partial protection in the DNA vaccine format.

However, when tested as a recombinant protein together with CpG, the results were inconclusive. Rather than giving up, we thought that these results might be due to the lack of a suitable vehicle, as it is known that molecularly defined antigens do not easily elicit the immune response. It is as if they were invisible to the organism because they are relatively small, soluble and unstable. Therefore, we revisited the previously mentioned liposome formulations and hypothesized that "encapsulating TR within this particulate system enhances vaccine efficacy". Since the immune system has evolved to recognize particles, such as viruses or bacteria, the idea of encapsulating the vaccine components in a particulate system to make the cargo "flashier" sounds reasonable. At this point, we thought, "This is precisely what our antigen needs!".

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Liposomes to the Rescue

Our selected protein, TR, was then encapsulated in liposomes and tested in mice as a vaccine alone or in combination with the adjuvant CpG. It yielded protection percentages higher than 80 % in several independent experiments. This showed us that the formulation plays a fundamental role in the efficacy of vaccines **(Figure 4)**. These results were so promising that we consider that the next step is to confirm that the immune system of people infected with the parasite recognizes this protein. If so, our vaccine could be produced under strict manufacturing conditions, and we could begin the clinical phases of development.

In perspective, the future of vaccinology will focus on particulate and molecularly defined vaccines. These are composed of a structured delivery vehicle, such as liposomes, and contain novel adjuvants, such as CpG's, and defined antigens: pathogen-specific proteins. Thus, these findings are evidence of a relevant and cutting-edge systematic research effort that could finally bring us closer to a vaccine against cutaneous leishmaniasis for use in humans. X

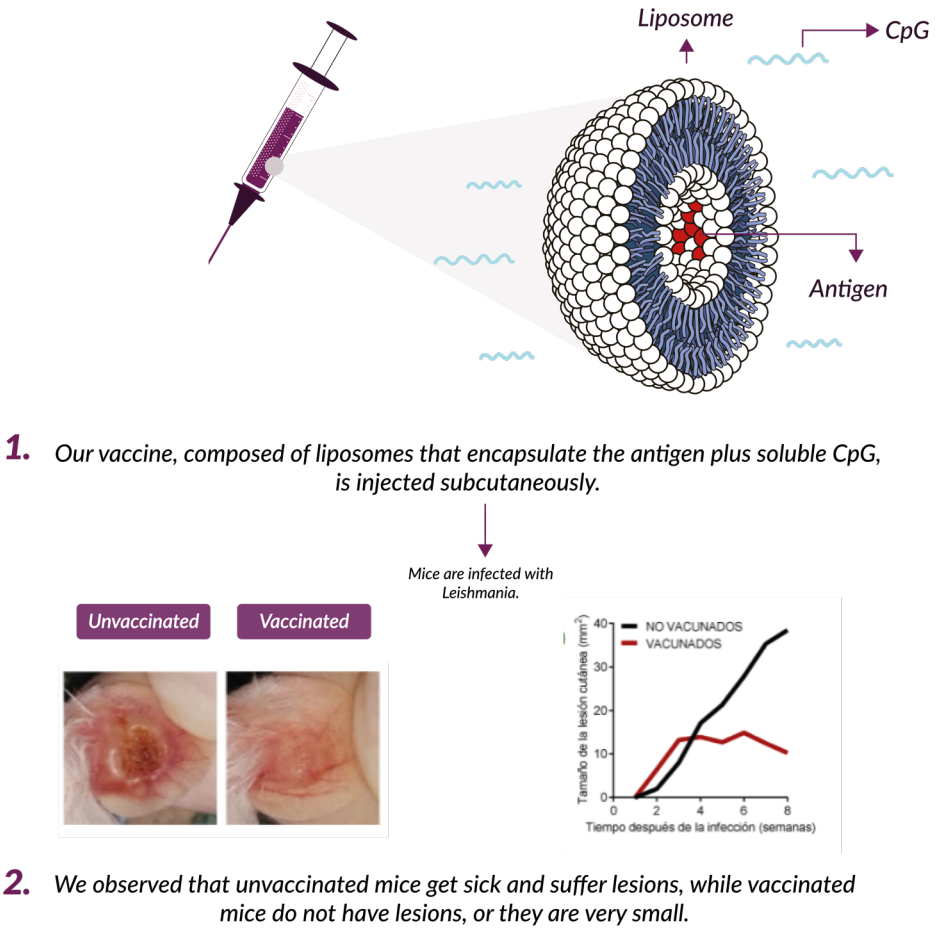

Figure 4. Our molecularly defined nanovaccine protects mice from leishmaniasis. Infography: David Bautista/ Carolina Gomes.

Glossary

Antibodies: soluble substances released by immune system cells (B lymphocytes) that help fight infection caused by a pathogen.
Cellular response: a type of response in which some cells of the immune system (lymphocytes) specialize in destroying invading pathogens by releasing substances toxic to them. They also signal to other cells to help control the infection.
Vehicle: in the context of vaccines, a vehicle is a colloidal particle or aggregate that is micrometric or nanometric and encapsulates or aggregates the other components of the vaccine.
Molecularly defined vaccine: vaccine composed of one or several antigens whose sequence or physicochemical characteristics are specifically known.

Neglected tropical disease: infectious disease prevalent in low-income countries or regions, usually in the tropics. They are neglected because they do not attract much attention from pharmaceutical companies and governments in the first world, so there is not much investment in scientific and medical research on them.

CpG: short single-stranded synthetic DNA molecule that activates cells of the immune system, as it is characteristic of the genetic material of viruses and bacteria. Therefore, the mammalian immune system recognizes it as a danger signal. In the formulation of vaccines, at specific doses, this substance functions as an adjuvant to activate the immune system.

Liposomes: small vesicles of lipids -fatty molecules- that are similar to cells. They even have the same type of membrane and can trap the vaccine components inside them or in their membrane. They help protect the antigen from degradation and enhance its activity (that is. they even act as adjuvants). They can also limit the negative effects of other components of the formulation.

Proteome and proteomics: proteome is the set of proteins in a sample. Proteomics is the science that studies the proteins in a sample through various molecular techniques.

Immunoproteome and immunoproteomics: immunoproteome is the relationship between the proteome and the immune system of an organism. Immunoproteomics is a set of techniques that help study this relationship. Among its purposes is the development of antigens to formulate vaccines.

Recombinant protein: protein that comes from a gene that, through genetic engineering, has been introduced into a cell of a species other than the one that originally produces the protein in nature. For example, the TR protein of the *Leishmania* parasite can be produced in a culture of *E. coli* bacteria.

Acknowledgments:

1. To the current and past members of GIM: Natalia Muñoz, Alexander Gómez, Jelver Sierra, Julián Londoño, Julio Jaramillo, Diana Colorado, Christian Piedrahíta, Cristian Salinas, Esteban Gómez, Lina Orrego, Verónica Guzmán, Paula Correa, Juan Camilo Álvarez, Diana Tabares, Jorge Tabares and Miguel Roldán.
2. To the collaborators: Tania Creczynski-Pasa (Universidade Federal de Santa Catarina, Florianópolis, Brazil), Patricia Cuervo (FioCruz, Rio de Janeiro, Brazil), Fanny Guzmán (Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile), Ana Mejía, Ómar Triana (BCEI Grup, Universidad de Antioquia), Juan Alzate and Gisela García (CNSG, Universidad de Antioquia).
3. To the funders: Colciencias/Ministry of Science, Universidad de Antioquia and Corporaciòn Universitaria Remington