

The background features several stylized virus particles in various colors (red, blue, orange, brown) with spike-like protrusions. A green DNA double helix is visible on the left side. The overall style is graphic and illustrative.

The increased production of vaccines

Butantan develops technology to increase production and lower costs

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With the capacity to produce 20 million doses, the flu vaccine plant at the Butantan Institute became fully functional in March and should ensure Brazil's self-sufficiency in the prevention of influenza in the elderly in 2012. This plant was inaugurated in 2007, but the plant's processes were validated only recently. In three to five years, the country could potentially become an exporter of influenza vaccines. Two technologies developed by researchers at Butantan promise to increase production without the need to expand the plant or purchase more raw materials. Each dose requires the use of a fertilized chicken egg for the reproduction of the virus, and the plant uses 20 million eggs per year to produce viruses. One of the new technologies permits the whole virus to be isolated, including all of the proteins. Current vaccines against the influenza virus are based on one protein, hemagglutinin A. The remaining proteins are discarded, but they can also provide protection. In addition, these other proteins accumulate fewer mutations over time than hemagglutinin A. "When we produced this new vaccine, we found that production per egg increased from two to seven times, depending on the serotype of the

virus," says Isaias Raw, a researcher at the Butantan Institute.

The second technology, already patented by the institute, allows for the isolation of an adjuvant substance, monophosphoryl lipid A (MPLA), which enhances the body's immune response by stimulating the production of appropriate antibodies or lymphocytes. Other adjuvant substances have already been created, but they are expensive or inaccessible. MPLA, curiously enough, is the byproduct of another line of research by Butantan that involves the development of a new type of pertussis vaccine that is considered safer due to the removal of lipopolysaccharide (LPS), which causes inflammatory and toxic reactions, from the bacteria. "We convert pounds of LPS into MPLA, which in small quantities can increase the responses to several vaccines so that they can be used in lower doses," says Isaias Raw. Tests on mice showed that MPLA can allow the vaccine to protect against influenza using just a quarter of the currently used dose. This effect has already been demonstrated in human beings.

This substance is being tested against various diseases. Butantan demonstrated that MPLA has the potential for use in canine leishmaniasis vaccines. These vaccines can break the transmission cycle

of the disease, which can be deadly to humans. MPLA is also the subject of investigations by researchers at the Ludwig Institute in New York, who seek to enhance the effect of a vaccine against ovarian cancer, and researchers from Ribeirao Preto, who are working on a vaccine against tuberculosis. "Soon, it will also be evaluated with a hepatitis B vaccine that is now ineffective in people over 50 who are awaiting kidney or liver transplant," says Isaias Raw. By increasing the immune response, MPLA also makes the vaccine less specific, and cases in which a flu vaccine immunized against serotypes not included in the vaccine have already been detected. MPLA is also inexpensive. "We can produce enough MPLA to manufacture 1 billion doses at a cost of just pennies. This makes Brazil immune to pressure from large pharmaceutical companies that do not want to sell the adjuvant but only the complete vaccine itself," says the researcher. The results, which must be validated in new clinical trials before the vaccine reaches the production line, were published in the journal *Vaccine* in an article by Raw, Cosue Miyaki, Wagner Quintilio and Eliane Miyaji, among other researchers at the institute. "The research does not end with the publication of the article but with the produc-



tion of vaccines to meet public demand,” says Isaias Raw.

Butantan believes that both technologies have the potential to increase the plant’s production capacity for the influenza vaccine from 20 million to 160 million doses. Such growth, after the completion of clinical trials, will naturally depend on market conditions, which have varied greatly in recent years. The plant was designed in 2004, when avian flu was a threat. Caused by the H5N1 virus, the flu outbreak in 2005 resulted in the death of thousands of birds and hundreds of humans in Vietnam, Thailand, Indonesia, China, Egypt and Cambodia. At that time, an old building at the Butantan Institute was converted into a pilot laboratory to start experimenting with small-scale production of the influenza vaccine. Simultaneously, funding was obtained from the state of São Paulo to build a plant and from the Ministry of Health to import the necessary equipment. The technology for vaccine production was transferred to the Charles Merieux laboratory, now called Sanofi-Pasteur, and is based on the reproduction of the virus in fertilized chicken eggs.

The picture changed radically in 2009 with the emergence of the H1N1 virus,

The WHO estimates that the alarms sounded regarding the severity of H1N1 were exaggerated, but then this organization launched a joint effort to provide vaccines.



which causes swine flu. This virus appeared in the United States, infected thousands of Mexicans, and quickly caused a pandemic. The pandemic strains were similar to those that caused the 1918 influenza pandemic, the so-called Spanish flu that killed 40 million people. Both viruses primarily affect young children and pregnant women, a sector of the population that was not previously targeted by vaccination campaigns. Suddenly, the demand for immunization increased 10 fold. The World Health Organization (WHO) now believes that the degree of alarm regarding the lethality of H1N1 was exaggerated, but at the time, the WHO launched a joint effort to provide vaccines. “The WHO had to prepare a vaccine and give it to producers, including the institute. There would not be vaccines for everyone, but the agreement between the Butantan Institute and Sanofi guaranteed the priority of acquiring vaccines manufactured overseas and allowed the immunization of some 80 million people,” says Isaias Raw.

Even before the application of new technologies, Butantan may be able to export influenza vaccines to countries in the Northern Hemisphere. Today, the productivity of the plant is seasonal. Manufacturing begins around September, when the WHO defines the three types of flu virus that are most prevalent in that period and sends samples to the manufacturers. For Butantan, the level



The production of the vaccine is based on the use of fertilized chicken eggs.

of production is high until April, when actual vaccination begins, and then the plant suspends all activity until the following September. “In this period, we could produce the combination of vaccines of the Northern Hemisphere and supply them to people who live above the equator in Brazil as well as in countries like Venezuela, Colombia and Guyana,” says Isaias Raw. “Today, immunization arrives too late in these regions and is not really effective.”

VIRUSES AND EMBRYOS

It took 7 years to complete the factory because it was necessary to overcome a series of obstacles. In addition to bureaucratic issues related to choosing a construction company for the project and importing special equipment, such as centrifuges, it was also necessary to develop a machine that destroys what is left of the eggs after the liquid full of viruses has been isolated. This leftover material has to be reduced to a powder to be safely transported and incinerated. This material must be destroyed to prevent it from becoming food for birds and other animals – a few live viruses would be sufficient to accidentally spread the disease. “It is a very complex process, which involves a specific technique for injecting the virus into each egg, separating the liquid in which the virus reproduces, and then purifying and re-purifying it to allow the environmentally friendly treatment of the waste material,” says Hernan Chaimovich, superintendent of the Butantan Foundation. The vaccine immunizes

“This giant step forward has made us (Brazil) the only country in Latin America that is currently producing influenza vaccines,” said Jorge Kalil.

against three types of influenza, but the production process is concentrated on one strain at a time. Before moving to the next strain, the plant must stop all activity for a few days and undergo a rigorous disinfection process. Sanofi monitored the production of the first batches and testified that the plant had been validated within the rules of the European Union. Investments for the implementation of the project exceeded R\$100 million, with funding from the state government of São Paulo, the

Ministry of Health and the Butantan Foundation. “These figures become irrelevant relative to the millions of dollars in savings that Brazil will realize when it does not need to buy products from international laboratories,” said Jorge Kalil, director general of the Butantan Institute, in an article published by *Folha de São Paulo* newspaper. “This tremendous advancement in science and technology turned Brazil into the only country in Latin America that currently produces influenza vaccines.”

GLOBAL ALLIANCE

The vaccine production capacity of public institutions in Brazil caught the attention of the Bill and Melinda Gates Foundation, which has also made contact with institutions in other countries. Last year, Butantan was visited by Tachi Yamada, president of the Global Health Program of the Gates Foundation, who was interested in learning more about the production capacity of the institution in São Paulo. The Gates Foundation, a charitable organization started by the founder of Microsoft, is seeking partners to produce vaccines at a low cost so that these vaccines can be provided to developing countries. A proposal for collaboration with Butantan, the Serum Institute of India, and Bio-Manguinhos in Rio de Janeiro was recently formalized. The partnership aims to produce approximately 30 million doses of a pentavalent vaccine against diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* B (which causes meningitis and other diseases). Butantan delivered a proposal to provide 100 million doses at \$1.50 each, which is scheduled for delivery in 2014, and this proposal is currently awaiting approval. The partnership of the Bill and Melinda Gates Foundation with the Global Alliance for Vaccines and Immunization (GAVI) has already produced remarkable effects in its search for new suppliers. Last month, four pharmaceutical giants, GSK, Merck, Johnson & Johnson and Sanofi-Aventis, agreed to sell GAVI vaccines against diarrhea and rotavirus at cost. This represents a 70% reduction in the retail price of the vaccine. Furthermore, two companies in India, the Serum Institute and Panacea Biotech, committed to providing the pentavalent vaccine at just \$1.75 per dose. ■