ART: HÉLIO DE ALMEIDA ON PHOTOS BY EDUARDO CESAR

GENETICS

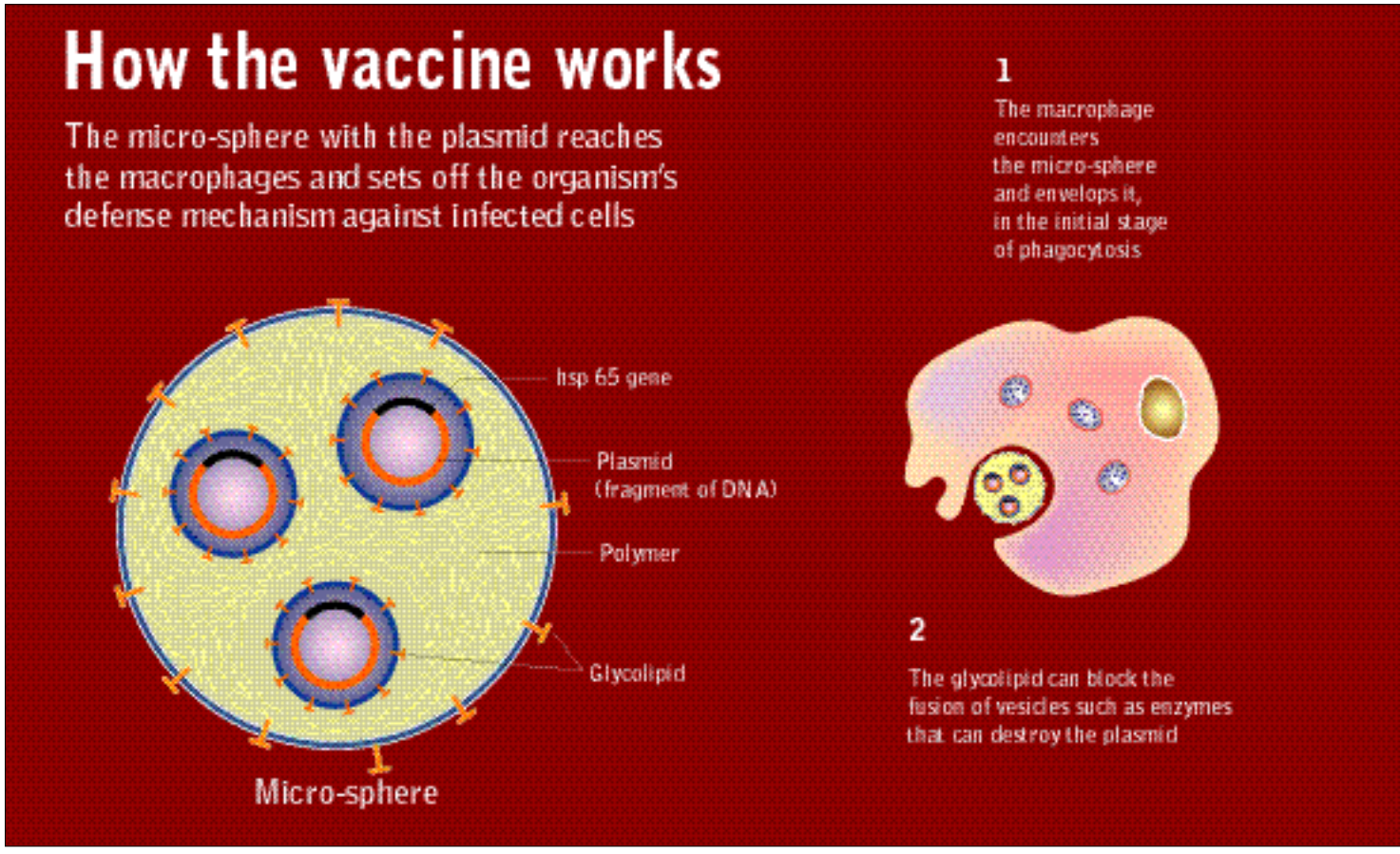
The target is human health

Tests are beginning on human beings of the first genetic vaccine developed in Brazil that in the laboratory has shown itself to be effective against cancer and tuberculosis

CARLOS FIORAVANTI

In three months at the most, a decisive chapter in the history of the first genetic vaccine developed here in Brazil will begin: tests will be carried out on human beings. The formula created and perfected over a ten-year period by the biochemist Célio Lopes Silva, from the Medical School of the University of São Paulo (USP) in the town of Ribeirão Preto, originally for the prevention and treatment of tuberculosis, will be given to a restricted group of eighteen people, all with an advanced phase of head and neck cancer that is no longer responding to any other treatment. In this phase, the completion of which was approved in August by the National Council of Research Ethics (Conep in the Portuguese acronym), the intention is to evaluate if the compound shows toxic effects, as yet not verified in laboratory animals, what is the best dosage, and if it really can contain the advance of the tumors. "The results, if positive, will serve as guidelines for other clinical studies (on humans) of this genetic vaccine", Silva comments.

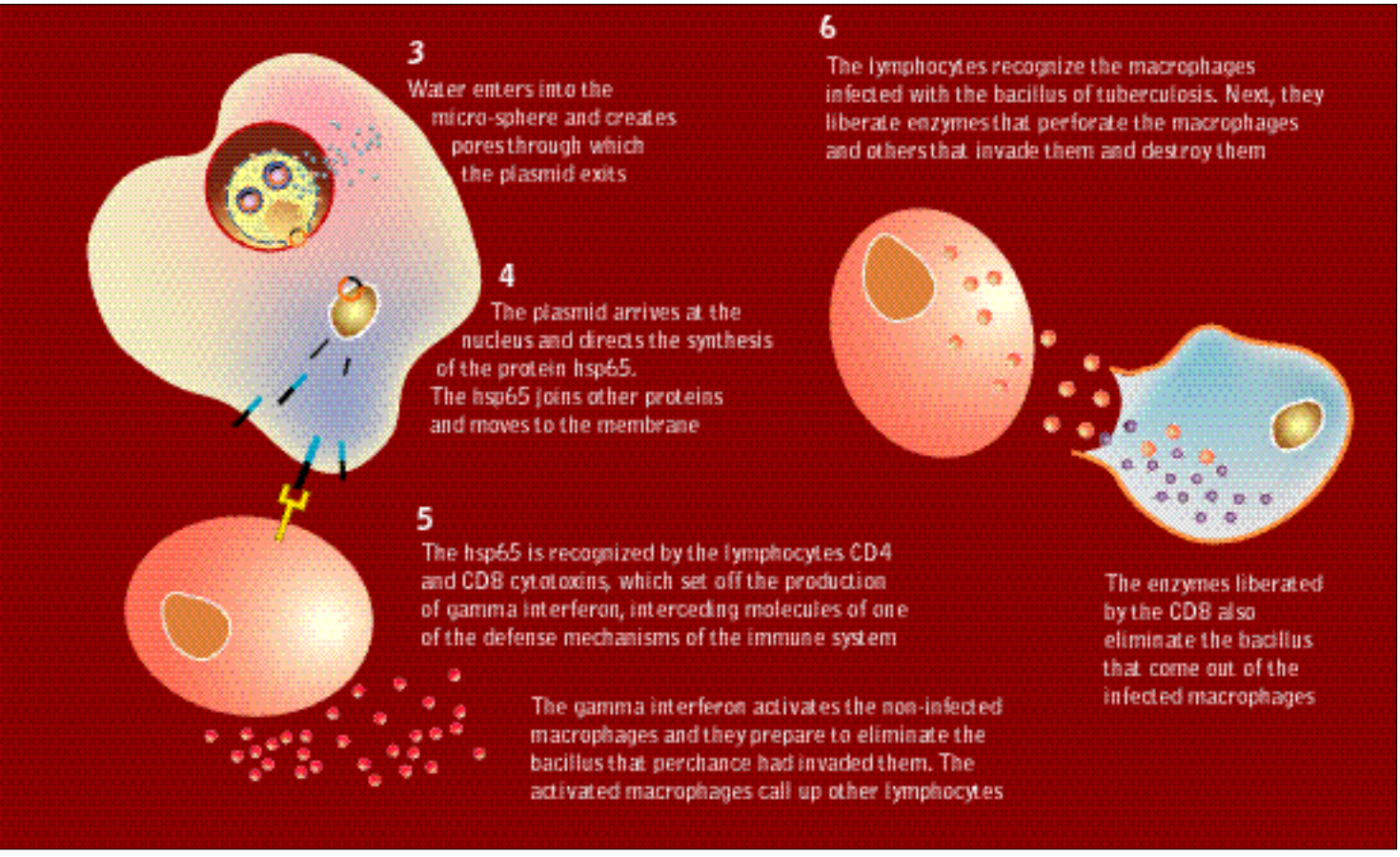
Planned in detail throughout this year, the next research phase may represent the most concrete evidence that this DNA vaccine, as it is also called, works like a medicine, acting when an illness has already installed itself within the organism, and not just like a traditional vaccine, of a preventative character. In fact, the most recent studies in this area have widened the meaning of the word vaccine, which today no longer represents something that avoids an illness, but also something that can cure an illness. The results, as they come forward, probably after some six months from the start



of the tests, could help to make possible a new pathway in the treatment of cancer, with fewer undesirable side effects than the traditional radio and chemotherapy treatments. In other countries, there are clinical tests underway with genetic vaccines against Aids, hepatitis and malaria, as well as various types of cancer. According to Kald Ali Abdallah, a researcher at the Medical School of USP in São Paulo and one of the two tests coordinators, there are no reports that serious side effects have been noted beyond a moderate fever and swelling at the location of the vaccine application, typical of any vaccine.

The future tests are also creating an expectation for another reason: if they are successful, they may prove that the formula over which Silva has been working intensely for the last ten years, has a range of action greater than that initially sought after: to protect against *Mycobacterium tuberculosis*, the bacterium that causes human tuberculosis, transmitted through the air, which every single second infects a human being. Installed in one third of the world's population, above all in the poorest countries, the disease annually kills two million people – just in Brazil there are 130,000 new cases per year. Preliminary results obtained with laboratory animals indicate that the vaccine, by way of small variations in the composition and the dosage, could be effective not only against tuberculosis, but also against other types of cancer such as that of urinary bladder and skin, as well as leishmaniasis and in animals, against bovine tuberculosis.

Besides the liberation for the tests on human beings, the researchers at the genetic vaccine laboratory, coordinated by Silva, have managed to produce a new form of the vaccine, which makes possible the application in a single dose – thus simplifying the work of medical doctors and veterinarians – and no longer in three applications as in the previous version. With the new formula of the vaccine, one dose ten times less potent than that previously adopted shows the same protective effect against the bacteria that cause tuberculosis, according to the results published this month in Gene Therapy, one of the most important international magazines in this area. Both in the old formula, with the pure DNA, and in the most recent version, in which the DNA can be found involved with other molecules, the vaccine prepares the organism so that it itself solves the problems or, in a more precise manner, works as a regulator of the immunological system – an immune-modulator. That is the reason why, in principle, it can be applied to more than one illness, to prevent or to cure. One of the most important abilities of the vaccine being studied by the Ribeirão Preto group is to act directly on the macrophages, cells that are essential to the defense system, which coordinate the



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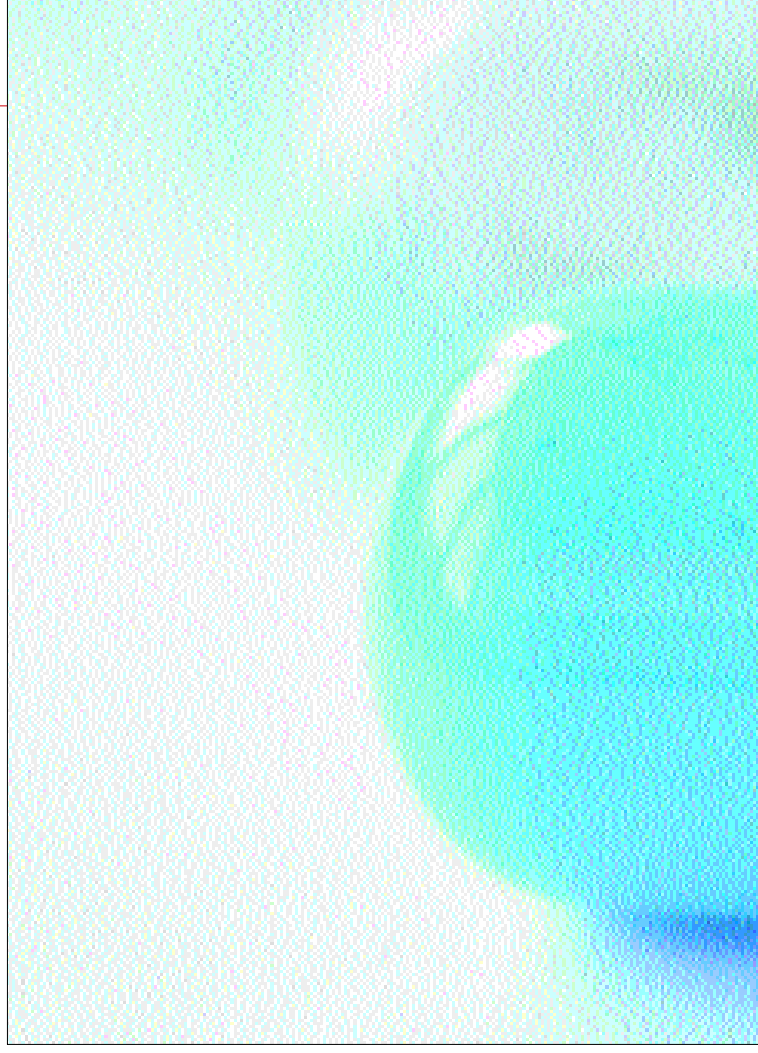
actions of other cells – it is exactly in the macrophages that the *Mycobacterium tuberculosis* install themselves and remains dormant until it later enter into action at a moment of the organism's fragility. By acting upon the macrophages, the DNA vaccine induces the production of molecules called gamma interferon, which regulate one of the types of responses of the immunological system against reagents foreign to the organism or against tumor cells, besides stimulating the lymphocytes that eliminate the infected cells (*see illustration*).

Although the new formula seems promising, the next set of tests are going to evaluate the efficiency of the simpler formula, with pure DNA – much more deeply studied – that will be applied directly on the tumor to be treated – the so-called epidermoid carcinoma, responsible for almost the total of nearly eleven thousand cases of cancer of the head and neck that arise per year in Brazil. Four teams from the Medical School of USP are going to be working during this next phase: two of them, coordinated by Abdallah and Jorge Elias Kalil, looking after the clinical evaluation of the selected individuals and following up any possible side effects due to the vaccine; and the other two led by Alberto Ferraz and Pedro Michaluart, will look after the analyses of the action of the compound directly on the tumor. "50 milligrams of the vaccine are sufficient for us to begin the tests", says Abdallah. But it is not so easy to obtain what may seem so little at the current stage of the research.

Silva knows that, in order to shortly begin the tests on human beings and to precisely define the true effects of the applications in other illnesses other than tuberculosis, he needs to find new sources for producing more vaccine. At least one thousand times more, jumping from the current milligrams to some grams per week, and with a degree of purity internationally recommended for human use, in such a way as to meet the conditions known as GMP (good manufacturing practices), that demand an extremely clean environment, with fewer particles in the air than that of a surgery room. "It's not easy", says the researcher from USP. "We now have to dominate the technology processes." The proportion among the reagents, the reaction time and the rate of production tend to change when the production leaves the laboratory bench and moves on to a larger scale.

At the beginning of October, shortly before flying off to India, on a federal government exchange mission in the research and production of medicines, Silva sent to the Ministry of Health the plan in which he outlines the necessary level of technological development. It is a project budgeted at R$ 20 million that includes the production and the testing of the DNA vaccine as well as other medicines against



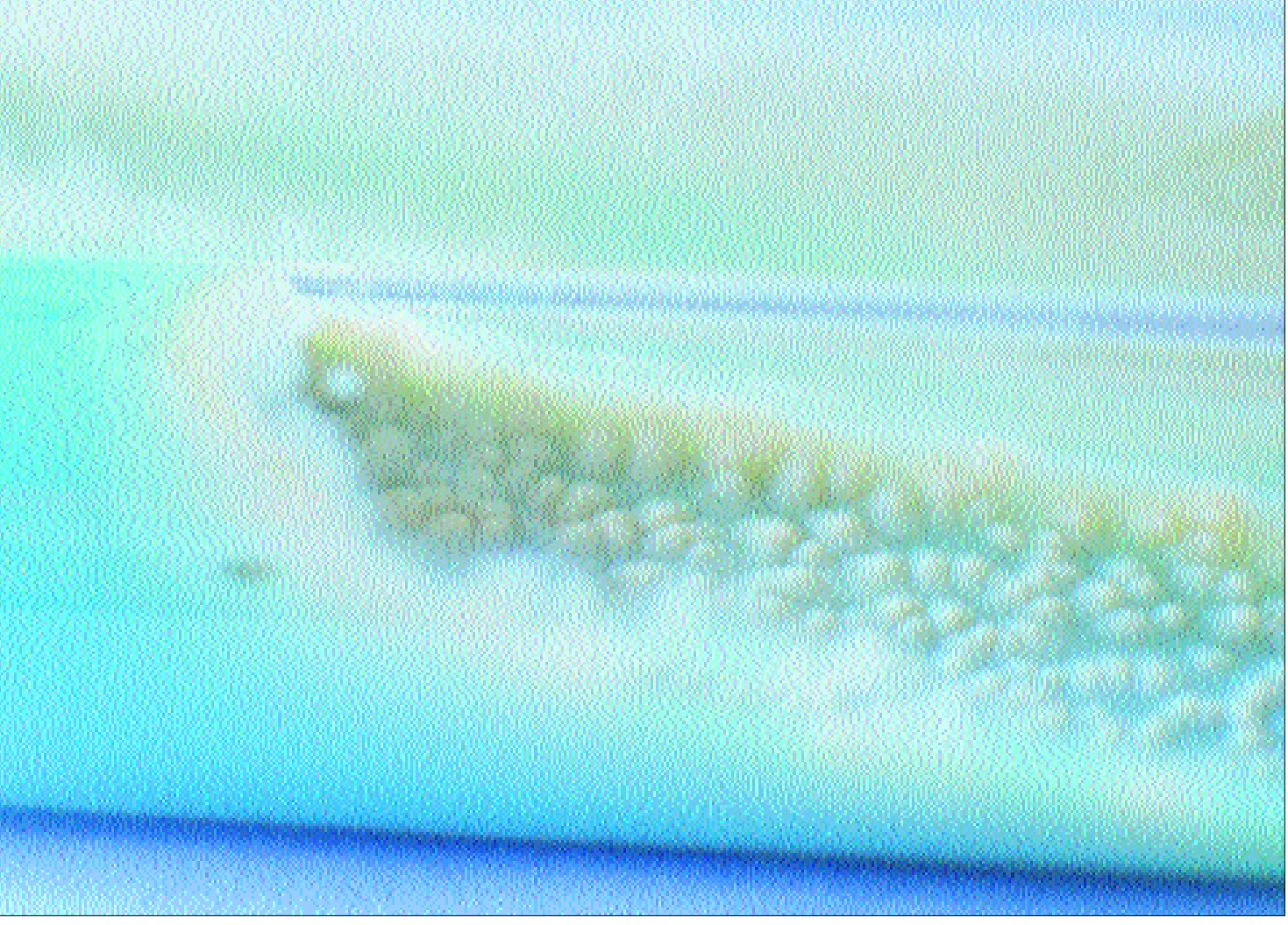
Mycobacterium tuberculosis cultivated in the laboratory: transmitted through the air, the bacterium kills two million people per year

tuberculosis, researched through the TB network, an association of specialists created last year to combat this disease. As a result of the integration brought about by this research network, the USP laboratory evaluated six hundred plant extracts for action against tuberculosis and verified that at least ten are also promising as potential alternatives against the bacillus known as Koch, referring to its discoverer, the German bacteriologist Robert Koch (1843-1910, Nobel Prize winner in 1905).

For the time being, Silva is waiting for some space to be freed up at the Medical School in order to set up the fermenters, centrifuges, extractors and purifiers for the DNA and to begin production on a pilot scale, with a productivity rate of up to one thousand times greater than that currently being undertaken. When the equipment start to operate, probably at the start of next year, the research born in the university will begin to turn into a product. At that stage there will be the participation of a technology based company, RDBiotec, established in May exactly for the purpose of increasing the scale of production, under the best possible conditions of time and cost, by way of a project financed through FAPESP. One of the RDBiotec directors is the researcher from the state of Minas Gerais José Maciel Rodrigues Junior, who at the end of last year left his post as professor of pharmaceutical technology at the Federal University of Minas Gerais (UFMG) so that he could dedicate himself full-time to the challenge of making viable Célio Lopes' vaccine, with whom, in truth, he had already worked for some years. It was professor Rodrigues who proposed, during 1997, the pathway that would lead to the current formula of the vaccine. It is from the new laboratory that the vaccine to be applied during the tests on human beings will come, beginning with cancer since there are already teams and adequate installations. Evidently, Silva is also contemplating on tests against tuberculosis, but he knows that they will be more complex: they demand an infrastructure and safety conditions that are more refined, so as to avoid the transmission of the bacillus resistant to any known medicine, which cause multi-resistant tuberculosis, against which the vaccine would initially be applied. If it works on the most serious strain of the illness, which in Brazil hits close to 1% of those who are infected, it will be almost certain that the DNA vaccine from the Ribeirão Preto group will be effective against the lesser forms of tuberculosis and will set itself up as an alternative vaccine to that most commonly in use against the illness, BCG (Bacillus Calmette-Guerin), which has been losing its effectiveness since in began to come into use way back in 1921.

Versatility - The in-depth studies into the bovine vaccine, already planned with researchers from the School of Veterinary Medicine of UFMG, will also depend on the supply of more vaccine. "The first tests suggest that the vaccine can stall the action of any *Mycobacterium*", Silva reports. Thus, the sacrifice of animals could be avoided, a policy currently recommended by the Ministry of Agriculture when bovine tuberculosis is detected, caused by *Mycobacterium bovis*. As yet without a cure, this illness affects almost 14 million animals of the total national herd of around 170 million head of cattle. According to Rodrigues, there are also promising results in the fight against leishmaniasis: in a test carried out with a research group from the Federal University of Rio de Janeiro (UFRJ), with mice that received the vaccine – by way of their nose – it managed to prevent them contracting this illness caused by the protozoa of the gender *leishmania*.

In the patenting process here in Brazil and abroad, the new formula of the vaccine contains three basic components. The first is the gene that contains the recipe for the production of the protein named hsp65 (hsp means heat shock protein and 65 indicates its molecular mass of 65 kilodaltons; the dalton



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is the unit of atomic mass). Produced by the bacterium under stressful conditions – for example, on the invasion of an organism –, this protein functions as an antigen, a molecule that brings into action the replies of the immune system, in this case, against the bacillus. It was with this gene, integrated into a plasmid (fragment of DNA in the form of a ring) that Silva obtained the preliminary results of the first genetic vaccine against tuberculosis in the world, announced in 1994 at a congress of the World Health Organization (WHO) in Geneva.

In the current version of the vaccine, the gene finds itself surrounded, firstly by molecules of a glycolipid – a sugar associated with fat (lipid) – trehalose dimycolate, incorporated as an assistant of the vaccine as it is found on the external wall of the micro-bacteria and it sets off the defense mechanisms of the immune system. Both the gene and the glycolipid occupy the cavity within a polymer (molecule with the same structure repeated a large number of times) know as poly (L-glutamic acid) or PLGA for short. The polymer forms micro-spheres of around three micrometers (a micrometer is one millionth part of a meter) – it was this material that Rodrigues suggested Silva incorporate into the vaccine some five years ago. Already produced at Ribeirão Preto, the micro-spheres lead the gene directly to the target – in the case of tuberculosis the infected macrophages – and, apparently, they also protect the DNA from attack by enzymes that normally would degrade it as soon as it would enter into the cells.

The new formula also had some factors found by chance. In 1998, still in search of the pathway that would lead to the most recent conquests, Silva discovered in the magazine *Vaccine* an article by Japanese researchers who had made use of glycolipids in association with the recombinant virus of hepatitis B. He hardly had to study anything in order to adopt this molecule, the properties of which he had detailed out some twenty years previously when he carried out his master's thesis at the Chemical Institute of USP, in São Paulo. Those were difficult times. Silva had been working as a chemical technician during the day, and make use of his free time to develop his master's thesis, and in the evenings he attended lectures. But these were not the greatest challenges of the student born in the rural district of Leme in the state of São Paulo. While still a child, the future author of the only proposal of a genetic vaccine against tuberculosis chosen by the WHO, cut sugarcane, picked corn and cotton, while helping his parents with the harvest. Later, as an adolescent, he worked as an assistant bricklayer and cloth salesman, but always without losing his

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Silva: clinical tests and new research with animals depend on a production rate one thousand times greater for the genetic vaccine

interest for study, which guaranteed him a place at USP, when eighteen. It was in 1990, already in a scientific career, that during his post doctorate carried out at the National Institute of Medical Research in London, he got to know about the gene of the hsp65 protein. He had no choice: it was the only one available in the laboratory and one of the few antigens of bacteria already cloned.

The gene that made Silva enter in the research into tuberculosis was in fact from another bacterium, named *Mycobacterium leprae*, which causes leprosy, and, as was later discovered, shows 90% similarity to that of *M. tuberculosis*. The research advanced, although the doubt persisted if it would not be better to make use of the equivalent of the bacillus of tuberculosis itself. Only recently was it discovered that the initial pathway was the one that was correct. In an article published in April in the magazine *Biochemistry*, Silva and Antonio Camargo, of the Butantan Institute, demonstrated that the gene that functions better is that of the protein of *M. leprae*.

Adjusted by Silva, Rodrigues and Karla de Melo Lima, one of the master's students supervised by professor Rodrigues at the UFMG and through Silva's doctorate at USP, this formula reduces by ten times the quantity of DNA of the plasmid: a single dose with 30 micrograms of DNA has presented the same effect as three doses of 100 micrograms of the DNA of the previous version, done only with the plasmid. The researchers believe that the tripod of gene, glycolipid and micro-sphere, could also work against other illnesses by exchanging the antigen, or by extending the immunological stimulus, using different combinations of mi-

THE PROJECT

Pre-Clinical Tests of Vaccines, Generic Therapy and New Drugs against Tuberculosis

MODALITY
Thematic project

COORDINATOR
Célio Lopes Silva – Medical School of USP in Ribeirão Preto

INVESTMENT
R$ 929,918.36 and US$ 846,938.75

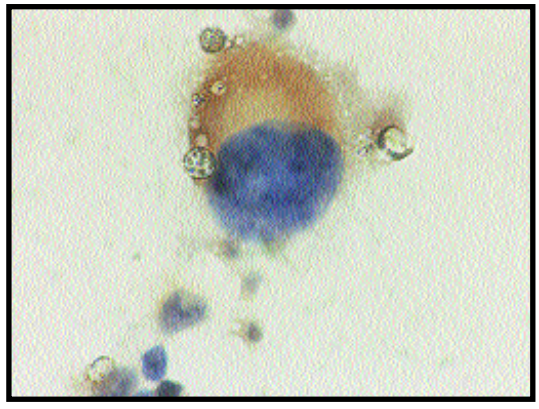
THE PROJECT

Production of Purified Plasmid DNA and Recombinant Proteins, on a Large Scale, for use in Vaccines and Diagnostic Applications

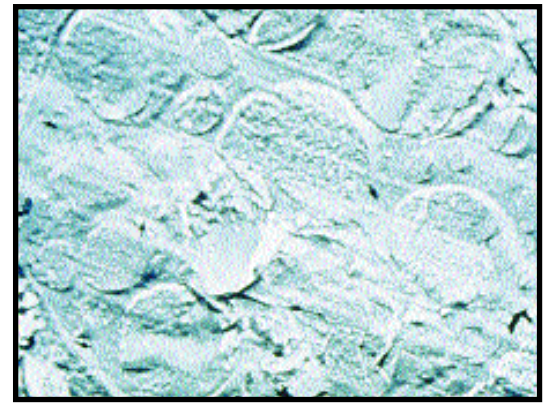
MODALITY
Partnership Program for Technological Innovation (PITE)

COORDINATOR
José Maciel Rodrigues Junior – Life Sciences Ltda.

INVESTMENT
R$ 21,000.00 and US$ 21,000.00

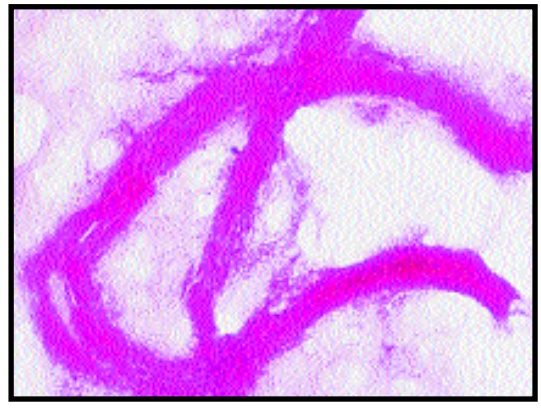


KARLA LIMA/USP



KARLA LIMA/USP

Macrophages envelop the micro-spheres (*details at the side*): advance against the *Mycobacterium* (*amplified one thousand times*)



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cro-spheres that release the DNA from time to time, in spaced out doses. According to Silva, the tests on mice demonstrate the action of the vaccine up to nine months after its application "Apparently", says Karla, "we can develop other strategies for the micro-spheres to degrade at different times, combining a faster action with one that is much slower."

Auto-immunity - Silva does not fear the risk of the gene impregnated into the vaccine that he has developed integrating itself into the genome of the treated individuals, as could occur in the classical occurrences of genetic therapy: at the end of September, it was noted that a medicine based on a retro-virus developed in France to treat a serious immunological problem, the so called blister illness, caused leukemia in a group of patients. "Even when used as a medicine, this vaccine does not present the same risks as the other genetic therapies", the researcher form USP says. "We have already proven that the gene does not incorporate itself into the genome of the people treated." His greatest worry is something else: the risk of the vaccine leading to an attack on the body itself, the so called auto-immunity effect.

In principle, the hsp65 gene of *Mycobacterium*, since it is similar (50% of similarity) to the one of the proteins of invertebrate animals in general, could generate a mechanism through which the immunological system could view its own body as something foreign – this is what happens in autoimmune diseases, such as arthritis, one of the types of diabetes and multiple sclerosis. The biochemist Alexandrina Sartori from the state of Rio Grande do Sul a researcher at the São Paulo State University (Unesp) of Botucatu, who did his post doctorate in Ribeirão Preto, has examined this possibility initially in arthritis, in a study conducted in conjunction with Rubens Santos Jr. and Marcelo Franco's team at the Butantan Institute.

The results could not be better: as well as avoiding the appearance of arthritis, the vaccine combated the illness previously active. In the first experiment, only one of the twenty five mice developed arthritis, induced by way of a mineral oil called pristane, which makes the infirmity appear on the joints on half of the animals to which it is applied. In a second test, the researchers verified that the vaccine made the arthritis disappear in all of the thirteen mice in which it had previously been installed. Alexandrina detected the research line that has now opened up: "If this vaccine also works on other autoimmune illnesses, it could be used in therapy, more than in immunization". Together with Ricardo Zolner's team from the State University of Campinas (Unicamp), the team from Ribeirão Preto is preparing to begin tests on another auto-immune illness, that of diabetes. The results should be published in the middle of next year. •