Perspectives of vaccination in Chagas disease revisited

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The perspectives for a Chagas Disease vaccine 30 years ago and today are compared. Antigens and adjuvants have improved, but logistic problems remain the same. Sterilizing vaccines have not been produced and animal models for chronic Chagas have not been developed. Vector control has been successful and Chagas incidence has come to a halt. We do not have a population candidate to vaccination now in Brazil. And if we had, we would not know how to evaluate the success of vaccination in a short time period. A vaccine may not seem important at the moment. However, scientific reasons and incertitudes about the future recommend that a search for a vaccine be continued.

Key words: vaccines - animal models - vector control - epidemiological targets - recombinant vaccines

In 1982, Carlos Chagas Filho, then President of the Academy of Sciences of the Vatican, invited Zigman Brener and myself to present, at a meeting celebrating the historical rehabilitation by the Church of Galileo Galilei, a paper about the "Perspectives of vaccination in Chagas disease" (Brener & Camargo 1982). At one session of this memorable meeting, physicists and astronomers gathered to discuss the Big Bang and, at another, Black Holes. At our session, researchers were asked to present their views on vaccines against parasitic diseases. After the seminal work of Ruth Nussenzweig on the protective potentialities of the circumsporozoite protein of plasmodia, we were quite confident that antiparasitic vaccines would soon be produced. These were days of great hope and optimism, and optimistic we were, my late friend Brener and I.

However, to avoid uncritical optimism, before writing our paper we decided to ask ourselves a few questions and to answer them impartially. I jotted down and kept our preliminary reflections. The paper presented at the Vatican session did not follow the order of the script. We were not bound to that script. We were simply asking and answering the questions that we candidly formulated to ourselves as a brainstorming exercise, not as a paper draft. Now, with three decades gone by, I think that it is worth comparing the "perspectives" as we saw them in 1982 and as I see them now. The questions that we formulated to ourselves were**:**

Question 1 - Are we going to have a vaccine against Chagas disease in 20 years' time?

The answer was no. Not then, not now. We submitted at the meeting that the logistics involved in finding protective antigens and to proceed from them to produce and test a Chagas vaccine in field conditions (see Ques-

tion 3) would require more than 20 years, even if we already had a "miracle antigen" at hand. Unfortunately, to complicate the matter of a Chagas vaccine, we were disappointed by the fact that a much acclaimed and supposedly protective surface protein of *Trypanosoma cruzi* had just turned out to be an artefact, not the acclaimed "miracle antigen" (Camargo et al. 1982). Three decades later, no such "miracle antigen" of *T. cruzi* has been proposed and no way has been found to shorten the time necessary to test a vaccine in humans. We are where we were 30 years ago.

Question 2 - If the vaccine is not coming soon, what could we do in the meantime to fight Chagas disease?

We summarily dismissed clinical treatment as a form of controlling Chagas. We also did not think that transmission through blood transfusion was an unsolvable problem and anticipated that legislation and technical improvements would bring transfusive Chagas to a halt. This is happening (Moncayo 2003). As for vertical transmission between mother and newborn, we could not foresee a solution. I still cannot. In summary, for the overall control of Chagas transmission, we undoubtedly favoured the control of its main vector in Brazil, the domiciliated *Triatoma infestans*. We were enthusiastic about the perspectives of insecticide-based vector control after the results of a large-scale program pioneered by the state of São Paulo in the 1960s. In the 1970s, the scientific community battled for the implementation of a similar program on a national scale. At the Caxambu meetings, Brazil, A Prata, JR Coura, JC Pinto Dias and Z Brener, to mention a few, insisted on the need to control *T. infestans* as the best way available to control Chagas disease. Carlos Chagas Filho and other scientists interceded with the government. Documents and petitions circulated between scientists and government. I remember having given an interview to a Brazilian magazine of wide circulation saying that, with the money that the government used to buy one fighter jet, we could control Chagas disease. Well, the interview did not please the military but, as a sign of the changing times, they did not harass me as I expected. In fact, to be fair, the most lucid among them were already committing themselves to

launch such a Chagas Control Program. In 1982, while we were writing our paper, the Brazilian program for the control of *T. infestans* was still in its infancy, but its initial successes were quite encouraging. We stressed this point in the paper and time proved that we were right. The current results are unquestionable: *T. infestans* has been eliminated or placed under control in Brazil (Dias 1987, Silveira & Vinhaes 1999) and in the countries that adhered to the Southern Cone Initiative, except in Bolivia and in a few other South American foci (Schofield et al. 2006). Human transmission of *T. cruzi* by *T. infestans* in Brazil has been curtailed after 30 years since the launching of the program (Massad 2008). However, in 1982 we were afraid that the program could suffer interruptions, opening the way to a dreaded re-colonisation of domiciles by *T. infestans* (Dias & Schofield 1999). This did not happen. We were afraid that colonisation by sylvatic triatomines could follow as domiciles were vacated by *T. infestans*. This did not happen at a significant scale either, and wherever it began to happen it was rapidly circumvented. There was the potential danger that populations of sylvatic *T. infestans* living in the Bolivian valleys could invade human dwellings, jeopardising control programs in South America (Noireau et al. 2005). As of today, there are no signs of such a phenomenon taking place (Cortez et al. 2007). As to the danger of the development of significant triatomine resistance to BHC, the chemical industry was quick to provide alternative insecticides, such as synthetic pyrethroids with residual effects. After three decades, it became clear that insecticide spraying, housing improvement and strict sanitary surveillance were indeed the best ways to curb *T. cruzi* domestic transmission, which had historically been the principal source of cases of Chagas disease in Brazil (Dias et al. 2002).

Question 3 - In this scenario, would a vaccine be necessary?

Yes, for at least three reasons. First, vaccines are the gold standard of prophylaxis for infectious diseases; they should be available for every infectious disease including Chagas disease, irrespective of any other reason. In addition, like other scientists, Brener and I fully appreciated the tremendous scientific challenge posed by a vaccine against Chagas disease and were very well aware of the considerable collateral amount of knowledge that could be gained from this saga regardless of its outcome. Moreover, in 1982 we did not know the results of the vector control program so we considered a vaccine necessary and essential if such a program failed in the immediate, as well as in the remote future. These reasons still prevail.

However, there is a problem today that we did not completely formulate in 1982, although I raised the issue on later occasions (Camargo 1984, 2000).The problem is: to whom would a Chagas vaccine be destined? Which population would be the target for a Chagas vaccine?

If we had had a vaccine on hand in 1982 we would have known exactly which population to vaccinate. In the 1980s, in Brazil alone, there were 25 million people at risk of infection. There were five million chagasic people and 120,000 new cases a year. Seropositivity for

T. cruzi was 4.4% for the general population and 5% for children in the 0-4 years age group. Chagas disease in Brazil was present in an area of more than three million km^2, including more than 2,400 municipalities. Today, except for the punctual explosions of oral infection cases (see item 5), there are no reports of new acute cases of *T. cruzi* infection. Seropositivity in children has dropped to 0.12%. The number of *T. infestans* captured in domiciles all over Brazil dropped by 99.3% and, from the 2,400 municipalities formerly infested, no more than 100 or so remain as unimportant foci. There is no defined population at risk of infection (Dias 1987, Silveira & Vinhaes 1999, Massad 2008).

Thus, there is no population candidate for a vaccine in Brazil at the moment. However, overall in the Americas, there are an estimated 16 million people still infected with *T. cruzi* and 50-80 million at risk. I cannot predict what is going to happen in the areas at risk, but programs similar to the Southern Cone Initiative are being carried out in Central America and in the Northern cone of South America. Bolivia as well is committed to a triatomine control program. Based on past experience in Brazil, we can foresee the success of these initiatives but, just in case, it would do no harm to have a vaccine.

Question 4 - What should be the requisites of a vaccine against Chagas disease?

The 1982 paper was not intended to deal with vector control, but with a vaccine for Chagas disease. Therefore, we proceeded to analyse the requisites that a vaccine should have. Our conclusions have changed little since.

Primum non nocere - Thirty years ago there were serious concerns about antigen-induced autoimmunity and immunosuppression in Chagas disease. Accordingly, we expressed our fears about the potential inconveniencies of antigen-derived vaccines. Presently, although some controversy remains (Leon & Engman 2001, Kierszenbaum 2003, Tarleton 2003, Hyland & Engam 2006), it seems that parasite presentation of antigens, that is, parasite persistence, is necessary to induce a focal inflammatory response (Tarleton 2001). The matter is still unsettled, but scientists nowadays are less scared by the spectre of autoimmunity. Thus, my original strong concerns about antigen-induced disease have subsided somewhat, although the "allergic" myocarditis reported by Muniz (Muniz & Pena Azevedo 1947) after the inoculation of *Rhesus* monkeys with merthiolate-killed *T. cruzi* still causes me some unrest. This is because large parts of the arguments in favour of and against autoimmunity have centred on experiments in mice. Primates have never been the object of experimental studies.

Sterile immunity - As we said in 1982: "A vaccine which merely attenuates the acute phase of the infection - a procedure possibly acceptable for other infectious diseases - would be of questionable value in Chagas disease.". This was because there was no evidence that the mildness or severity of the acute phase was directly related to the onset and severity or mildness of chronic disease. Clinical records were full of reports on the lack of correlation between both phases (Brener & Andrade

1979). Mouse experimental models are inadequate to test this correlation, whereas rabbit, dog and monkey models have not properly coped with the problem. We were afraid that a few infective forms escaping a non-sterilising vaccine could become responsible for a full course of chronic symptoms. For this reason, until the contrary was proved, we considered the sterilising requisite as absolutely mandatory. However, as mandatory as this precept seems to be, to date, the success of vaccination attempts remains measured by the mortality rates of mice in the acute phase of a challenging post-vaccinal infection. Most authors ignore or give little significance to residual patent or sub-patent parasitaemias that may lead to chronic infections. It has actually been shown in humans that parasite persistence in tissue foci is necessary for chronic inflammation and disease (Vago et al. 1996, Anez et al. 1999, Tarleton & Zhang 1999, Zhang & Tarleton 1999). Unfortunately, chronic evolution has not been properly evaluated in experimental models mainly because the most common experimental model, the mouse, is inadequate for that purpose.

Effectiveness against all T. cruzi strains - In the paper to the Academy of Sciences of the Vatican we stressed the fact that there were diverse strains of *T. cruzi* and that a vaccine should be effective against them all. However, we did not know at the time the full extent of *T. cruzi* genetic variability. Ever since, isoenzyme phenotyping and spliced leader gene and ribosomal genotyping revealed a considerable and ever-increasing number of lineages and hybrids (Miles et al. 1978, 2003, Souto et al. 1996, Brisse et al. 2000, Fernandes et al. 2001, Marcili et al. 2009, unpublished observations). The antigenic makeup of these strains is unknown, whereas their immunogenic and pathogenic importance remains to be assessed. Is there a universally protective antigen? Until we know that, a question will remain unaddressed**:** would a vaccine produced against one strain be effective against all *T. cruzi* existing strains?

Question 5 - How to evaluate the effectiveness of a Chagas disease vaccine?

The experimental model - In 1982, we stressed that the mouse model obviously was not an adequate experimental model for Chagas disease. Three decades later, we still lack a widely adopted and satisfactory experimental model for Chagas disease. The mouse is a handy and useful experimental model, but in many systems it is used only for "first approximations" to be followed by tests in more adequate models. This is the case, for instance, of drug screening for effects and toxicity; tropism, infectivity and virulence of infectious agents; antigens, antigen adjuvants and deliverers, and preliminary antibody and cell immune responses. Unfortunately, for Chagas disease, the mouse remained the sole model throughout the years. In Chagas, mice fail to mimic chronic disease as they fail to recognise *T. cruzi* antigens that are important in the human immune response (Galili 1993, Galili & Andrews 1995). As we said before, and I submit here again**,** the dog is probably the best lab model for Chagas disease. In nature, dogs develop a

human-like Chagas disease, both acute and chronic, and are found naturally infected in the most diverse biomes colonised by man. For these reasons, they are excellent epidemiological sentinels for *T. cruzi* vectorial transmission (Umezava et al. 2009, unpublished observations). The dog model has only recently been more deeply explored (Valadares et al. 2008, Guedes et al. 2008) and it has been shown that dogs respond to *T. cruzi* infection with the full cohort of factors and kinins that accompany the human infection (Guedes et al. 2009). I believe that dogs would be the model of choice for testing prospective vaccines, although I agree that it may be an inconvenient model for first approximations because of handling and nursing needs and the emotional objections of bystanders and researchers alike.

Diagnosis, vaccination and cure - The laboratory diagnosis of Chagas disease was no longer a problem in 1982. Serological diagnosis was ripe for the national-scale epidemiological survey that preceded the launching of the Chagas control program in the 1980s (Camargo et al. 1984). However, serology could not distinguish between present and past infections. Ever since, efforts have been made to find serological (Krettli & Brener 1976, Krettli 1980) or molecular markers of cure, but the problem persists. Without a diagnostic method with such capabilities, the actual effectiveness of a vaccine cannot be properly evaluated, not only in experimental models, but also in humans. As we said in 1982 and I reiterated at later opportunities (Camargo 1984, 2000), due to the silent and slow-evolving pathology of chronic Chagas, the evaluation of the effectiveness of a vaccine would take years. Thus, even if we had already overcome all laboratory and experimental barriers to developing a heroic vaccine capable of attenuating the acute phase, the field tests designed to assess whether such a vaccine could prevent chronic disease in man would take decades of clinical observation. It would take at least two or three decades, in the favourable (although unfortunate) scenario that there was a population to vaccinate, as discussed in item 3. The situation has not improved since 1982 and it is dismaying to see that the molecular advances in the development of candidate antigens and vaccines have not been accompanied by the development of experimental models for Chagas disease, nor by methods to ascertain its cure.

Question 6 - What is new?

New techniques, new scenarios, new problems - In 1982, we registered 49 full papers on vaccination attempts published in regular scientific journals and found more than 150 reports in Brazilian and Argentine meetings alone. In these attempts, the antigens used were**:** "avirulent" or attenuated strains of *T. cruzi*; culture forms of non-pathogenic insect trypanosomatids; killed culture and blood forms and sub-cellular fractions and purified, cellular or cell-surface antigens of *T. cruzi* or other trypanosomatids. Such attempts may not have stopped but, apparently, editors have stopped publishing them. Exceptionally, one or another attempt with living trypanosomatids has reached the scientific press in re-

cent times (Basso et al. 2008). Despite many optimistic claims by authors, all vaccination attempts failed to confer sterile immunity. At best, they increased the survival rates or prevented the death of vaccinated animals after a *T. cruzi* virulent challenge. The animal model used was always the mouse, with three or four exceptions. Adjuvants were used sporadically. The vaccination and challenge routes were always sub-cutaneous or intraperitoneal in a few instances.

Things have changed in recent years. The DNA vaccine approach has taken over the scene. Initially, *T. cruzi* surface genes inserted in plasmids have been tested, but they also failed to induce sterile immunity in mice (Wizel et al. 1997, 1998, Costa et al. 1998, Boscardin et al. 1999). The choice of a plasmid stimulatory of Type I immunity as the vector for two surface genes induced total protection against a virulent challenge. The parasitaemia was null, but unfortunately the model used was the mouse, which provided information as a "first approximation" only (Machado et al. 2006). Proposed therapeutic vaccines represent a departure from the all-or-none protection model. Since DNA vaccines are in principle capable of producing antigens for the duration of the life of the vaccinated mice, they represent a life-long source of antigens that may induce protection or reduce the severity of the "disease" in mice, but only in mice (Garg & Tarleton 2002, Zapata-Estrella et al. 2006, Sanchez-Burgos et al. 2007).

An alternative form of vaccination, mucosal or oral vaccination (Hoft et al. 1996, Schnapp et al. 2002, Hoft & Eickhoff 2005), although tested with certain success in mice only, finds incidental support in the current epidemiology of Chagas in the sense that it may prevent mucosal penetration and proliferation of *T. cruzi*.

Presently, new acute cases of Chagas disease in Brazil result from blood transfusion, trans-placental transmission and oral infection. The latter form results from the accidental ingestion of grinded triatomines carrying *T. cruzi* originating from wild animals. Triatomines have been accidentally grinded with fruit in the preparation of juices of açai (a palm tree widespread in Amazonia) and sugar cane. Clearly accidental, these outbreaks of oral infection can occur anywhere in the country, but have been more frequent in Amazonia. The episodes usually strike dozens of people at once, are serious and often fatal, but are always limited in space and time. There have not been more than 200 or 300 such outbreaks (Umezawa et al. 1996, Cardoso et. al. 2006, Coura 2006, Aguilar et al. 2007, Roque et al. 2008, Valente et al. 2008).

Would an oral vaccine be of help in preventing these Chagas outbreaks? No. Even if it prevented mucosal infection it would be of no use as a vaccine. This is because, again, we cannot define the population at risk.

Finally, since vector control was effective in curbing Chagas transmission in Brazil and since vaccines cannot be used at the moment to prevent the current forms of Chagas transmission (transfusive, trans-placental or accidental), does this mean that a vaccine against Chagas disease is unnecessary?

No, it does not. It may be unnecessary for Brazil at the moment, but Chagas is still endemic in large segments of Central and South America where close to 100 million people are still at risk. Vector control programs are under way, but they depend on reliable sanitary services, economic resources and political resolve. This is not a trivial conjunction of factors and even where they already co-exist, political turmoil and economical crises may endanger any vector control program that relies on stability. In contrast, vaccinated people will remain immune through political unrest, economic fluctuations and the not-uncommon episodes of public health failure.

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