

Professor Ruth Sonntag Nussenzweig (1928-2018)

First steps towards a successful anti-sporozoite vaccine against malaria

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On Sunday, April 1, 2018, Professor Ruth Sonntag Nussenzweig, 89, died of pulmonary embolism in New York City, where she lived and had spent most of her life working on a vaccine against malaria. Despite her impaired mobility, the consequence of a fall, she remained committed to malaria research using non-infective sporozoites as the source of antigens. Two of the human vaccines presently in clinical trials are based on her original research.

Ruth was born in Vienna, Austria in 1928, arriving in São Paulo, Brazil in 1939 at age 11 with her parents Eugenia and Barouch Sonntag, both medical doctors, and a younger brother, who died in a tragic accident years later while working in a Kibbutz in Israel. Her family migrated to Brazil, fleeing the totalitarian regime in Germany to which Austria had just been annexed and where her father, a non-religious Jew, had been imprisoned. While her mother was waiting in line to visit her husband at the prison one day, she was identified by an Austrian Nazi who let them go, and the family escaped. They first went to Belgium, where Ruth spent most of her youth, and then to Brazil, where she attended school in São Paulo (**Figure 1 and Figure 2**). She studied medicine at the University of São Paulo (USP), and there she met her classmate, Victor Nussenzweig, who would become her life companion and collaborator. Like her, he came from a traditional Jewish family that emigrated from Poland to escape pogroms. Victor was born in São Paulo and was interested in social politics, serving as president of the students association and was a member of the communist party when they first met. Ruth soon convinced him to become a scientist instead of a political leader; she believed that it would be more helpful to



FIGURE 1: Ruth (arrow) as a young student in São Paulo.

humanity. Both started work as medical science students in the Department of Parasitology, USP, doing research with an initial common goal of finding a cure for cancer using *Trypanosoma cruzi*, based on a Russian paper cited in *Science*, wherein the authors claimed that a parasite endotoxin could cure cancer¹. With permission from Professor Samuel Pessoa, the group leader at the Parasitology Department (USP), Ruth and Victor started work with animals experimentally infected with *T. cruzi*. At the time, chronic human disease was highly prevalent in South America, including in São Paulo, with no available treatment and parasitological diagnosis was very difficult. Although they never found a cure for cancer, both learned a lot working in the laboratory with *T. cruzi*-infected mice and with human blood from Chagasic patients. By the time Ruth and Victor finished medical school, they had published several scientific papers on Chagas disease and leishmaniasis, among which two findings were of particular practical importance for human disease prophylaxis: I) modifying xenodiagnoses, a parasitological test to detect parasites in the blood obtained

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FIGURE 2: Ruth with colleagues as a medical student at *Universidade de São Paulo* and in her year of graduation (1953).

during chronic Chagas disease. Instead of feeding the insects by placing the blood-sucking laboratory bugs directly on the patient's skin, they blood-fed them through an artificial membrane, which was less unpleasant for the patients²; and II) demonstrating that the gentian violet stain destroyed *Trypanosoma cruzi* trypomastigotes *in vitro* upon incubation with infected blood from patients, which could then be used for transfusion with no risk of infecting other individuals³. For decades, the gentian violet method was largely used to avoid transmission of parasites via blood transfusion in endemic areas of Brazil and other Latin American countries. Both papers had a great impact on the control and prophylaxis of Chagas disease.

Ruth and Victor studied and wrote their papers at the library of the Department of Parasitology (**Figure 3**), which is where they chose to celebrate their civil wedding in 1952 (**Figure 4**). During this year, Ruth worked as a parasitology instructor for medical students at USP. In 1953, she finished medical school and became an assistant professor of Parasitology. The Nussenzweig couple went on a leave of absence to work in Paris, where Ruth was a Research Fellow at the Laboratory of General Biochemistry, College de France. After their return to Brazil in 1964, preceded by a brief stay at New York University, they found their old Department of Parasitology emptied by the newly installed military regime in the country. Professor Samuel Pessoa and other colleagues had been imprisoned or had left the country, and the new Department Chief was not friendly. The young couple (**Figure 5**), who already had three children, soon decided to leave the country again. Victor contacted Professor Baruj Benacerraf (Nobel Laureate of Physiology and Medicine 1980), with whom he had worked in former years, to ask if he could find a position for himself and Ruth at the New York University Medical Center (NYUMC). The Nussenzweig family moved to the USA, where they would spend the rest of her life. At NYUMC, she and Victor led brilliant and successful scientific careers working in different departments; Victor, an immunologist, stayed at the Department of Pathology.



FIGURE 3: Ruth Sonntag and Victor Nussenzweig, as students, at the library in the Department of Parasitology at *Universidade de São Paulo*.



FIGURE 4: At their civil wedding, in the library of *Universidade de São Paulo* with Prof. Samuel Pessoa (arrow), Head of the Parasitology Department.



FIGURE 5: Ruth and Victor were life companion and collaborators, and had three children all born in São Paulo.

Ruth began as an academic fellow in the Department of Pathology and, in 1965, she became an assistant professor in the Department of Preventive Medicine at NYUMC. She joined a group working on the biology of malarial parasites, successfully producing sporozoites of *Plasmodium berghei* in special acclimatized insectary with low temperatures. Ruth's first publication was in 1966, on the course of sporozoite-induced *P. berghei* infections in different hosts and lethal malaria in mice⁴. At a time when sporozoites were considered non-antigenic, she described protective immunity against sporozoites of *P. berghei* in the prestigious *Nature* Journal^{5,6}. All mice repeatedly intravenously immunized with X-ray-irradiated parasites produced high levels of antibodies and complete protection against a challenge with infectious sporozoites. The monkeys repeatedly vaccinated with weak sporozoites dissected from the salivary glands of the mosquitos also displayed antibodies as well⁷. A protective immunity in mice could also be produced by the bite of X-irradiated mosquitos infected with *P. berghei*⁸. By 1976, Ruth had published around 40 papers, several as a first author with the NYUMC group, and began to publish with her first PhD student fellows⁹⁻¹¹.

In 1978, Ruth returned briefly to São Paulo to present her NYUMC work to the Department of Parasitology, USP, receiving a doctorate degree. Back in New York, Ruth soon became full professor and head of the Division of Parasitology at the Department of Microbiology. She was chosen chairperson of the new Department of Molecular Parasitology in 1980, remaining the group leader for almost 20 years. Ruth was the first woman to occupy this position at NYUMC, where she became internationally renowned for her pioneering work on the malaria vaccine, attracting various researchers and post-doctoral fellows to NYUMC, as well as generations of students and many collaborators. Her work on malaria vaccines brought strong financial support from various international agencies.

In the next decade, Ruth's group demonstrated that anti-sporozoite antibodies were related to acquisition of protection against human malaria, since they occurred in high frequency and titers in individuals living in hyperendemic malaria areas of Africa¹², who were known to have acquired protection. Her group was the first to produce a monoclonal antibody against parasite antigens, published in the *Journal Science* in 1980¹³.

The anti-sporozoite antibodies caused inhibition of entry by sporozoites into cultured cells, and this is considered the first direct evidence of protective antibodies directed against the circumsporozoite protein (CSP), including against *P. falciparum* and *P. vivax*¹⁴⁻¹⁶. In the 1980s, her collaboration with Victor became intense once again, focusing on these vaccine projects.

A new approach towards the identification of mosquitoes infected with malarial parasites became possible through these monoclonal antibodies, as proposed by Fidel Zavala^{17,18}. The interaction of antibodies in this simple *in vitro* immunoassay allowed the species of parasites infecting the mosquitoes to be identified, and this was widely used in epidemiological studies throughout many parts of the world¹⁹⁻²¹.

Another breakthrough by the group was the cloning of the *P. falciparum* CS gene, which showed that CSP contained an interesting repetition of four nucleic acids, believed to be the antigen against which the protective monoclonal antibodies were directed²². Understandably, the group rushed into producing a synthetic antigen with these identified repeats and using it to immunize volunteers²³. The results, however, were somewhat disappointing. Most of the healthy medical school students receiving the synthetic antimalarial vaccine developed a low antibody response. Nevertheless, the CD4 lymphocytes from the immunized individuals were able to recognize the conserved repetitive epitope, important later in further development of the vaccine against malaria²⁴. Not surprisingly, the failure to protect vaccinated volunteers did not discourage Ruth, despite the fierce criticism she suffered from various scientific groups, including in the international media, which shocked most of us, her former disciples!

In subsequent work, her group clarified several other mechanisms involved in sporozoite-immunity against the early parasite liver stages that led to full protection, including through the use of other models. Together, we characterized the CS protein on the surface of *P. gallinaceum* sporozoites, a laboratory model my group developed at FIOCRUZ-Minas²⁵. Through collaboration with Fidel Zavala and Mauricio Rodrigues, the relative contribution of antibodies and CD4+ and CD8+ T cells to sporozoite-induced protection against malaria was clarified²⁶. They demonstrated that gamma/delta T cells contribute to immunity against the liver stages of malaria parasites²⁷. An alum absorbed, synthetic, multiple-antigen peptide system based on B and T cell epitopes of the *P. falciparum* CS protein was considered to have potential vaccine application²⁸. Indeed, the importance of adjuvants was further demonstrated during the human trials of a recombinant CS antigen of *P. falciparum*.

Soon after the NYUMC group published their initial results showing that mice could be fully protected after vaccination with sporozoites, successful vaccines were reproduced by different groups in human volunteers also immunized with X-ray-irradiated sporozoites repeatedly administered through hundreds of mosquito bites. Clyde's group worked in collaboration with the NYU group and was the first to put the protocol into practice. They showed that the vaccinated human volunteers were fully protected from a malaria infection when challenged with the homologous infectious sporozoites delivered by mosquito bites;

first against *P. falciparum* and soon also against *P. vivax*^{29,30} with the NYUMC group. But, curiously, Ruth did not co-author the paper, apparently because as a woman she could not enter the prison where the experiments were performed.

The first sub-unit anti-malarial vaccine produced against *P. falciparum* used in field trials was by the pharmaceutical company GlaxoSmithKline (GSK), using a recombinant antigen, RTS, S containing the CSP repeats of *P. falciparum* in the recombinant hepatitis virus^{31,32}. Before starting human field trials, the research leader at GSK proposed collaboration with the Nussenzweig's group, but they preferred to continue in academia, doing basic science. The GSK vaccine was protective when administered together with a special adjuvant, and although unable to prevent malaria in the immunized individuals, it prevented deaths among the vaccinated children. Considering the high level of infant mortality caused by malaria in several African countries, the World Health Organization (WHO) recently approved this vaccine for use in three African countries (Ghana, Malawi, and Kenya). This is the only malaria vaccine in phase IV of clinical trials³³.

Several decades after Ruth's initial vaccine work at NYUMC, another group successfully used the whole non-infectious sporozoites obtained from X-ray-irradiated mosquitoes shown to protect immunized human volunteers, as described by Steve Hoffman in Sanaria³⁴. His main objective was to mass-produce and deliver the sporozoites of *P. falciparum* (*Pf*spz) under strict conditions of safety, injected with a needle instead of the traditional mosquito bites. More recently, Steve's group was able to produce and harvest billions of *Pf*spz through laborious dissections of X-ray-irradiated mosquitoes, and cryopreserve them in vials for subsequent delivery through needle injection to volunteers in Mali, a highly endemic sub-Saharan African country³⁵. The protocol still needs to be made affordable for further use in hyper-endemic malaria areas, mostly in poor countries unable to afford even diagnosis and treatment or other control measures, such as mosquito bed nets.

Ruth published over 230 papers while at NYUMC, many in important journals, and wrote several book chapters and reviews about malaria and vaccines. For her outstanding work, Ruth received several medals and international awards. She served as a consultant for the WHO and was a member of international scientific committees like the Pew Foundation and the Royal Academy of Medicine of Belgium, among others. In recent years, she, Victor, and Youyou Tu, the recipient of the Nobel Prize in Medicine, received the Warren Alpert Foundation Award, from Harvard, valued at US\$500,000. In 2017, she received the Clara Southmayd Ludlow Medal of the American Society of Tropical Medicine and Hygiene. She was elected a member of the Brazilian Academy of Sciences in 1997, and of the National Academy of Sciences in the USA in 2007. Over decades, she contributed significantly to the training of numerous students and fellows, most working on a malaria vaccine, several of whom became internationally renowned researchers in many parts of the world.

Ruth always believed in a malaria vaccine, although aware of the need for more accessible techniques to improve its

effectiveness. She became especially interested in *P. vivax*, a neglected parasite that causes a type of human malaria less severe than *P. falciparum*, but responsible for the most frequent malaria in the world outside of Africa, including in the Amazon Region. In her later years, Ruth worked on a new presentation of the antigens, aiming for a vaccine with *P. vivax* sporozoites, a collaborative project with the late Prof. Mauricio Rodrigues, then a younger researcher in the School of Medicine at Federal University of São Paulo [*Universidade Federal de São Paulo (UNIFESP)*]³⁶. *P. vivax* malaria, although less lethal, is more common in Latin America and is a difficult parasite to work with, since the blood forms are not maintained in long-term cultures, making it difficult to infect the mosquito host. Not long ago, Ruth and Victor also started an ambitious project supported by Fundação de Amparo à Pesquisa de São Paulo (FAPESP), which included a travelling grant that allowed them to return three times a year to São Paulo, working with groups at UNIFESP and USP. Parts of the results from this project were published, and her last paper came out early in 2018³⁷.

My affection and respect for Ruth began at our very first meeting, in early February 1972, on a harsh winter morning after a blizzard in New York City (NYC). She came to rescue me in the area reserved for passengers at JFK International Airport. I had in hand an unusual traveling companion, a small monkey (*Saimiri sciureus*) captured on the outskirts of São Paulo by the great expert of simian malaria, the medical epidemiologist Dr. Leonidas Deane, her old friend and a Professor at USP. I had problems trying to board at the Galeão airport, prompting a long negotiation between the flight attendants and the WHO international health officer, a medical epidemiologist working for the Pan-American Health Organization (PAHO). He was able to convince the airline personnel of the vital importance of the monkey in the future of a human malaria vaccine, without which I could not board, he argued. Upon arrival in NYC, the providential presence of Ruth was a must, at our first meeting! I consider it a great fortune to have worked with her over the following years at NYU, and several times afterwards. Ruth introduced me to the international malaria community during my first experience abroad, allowing me to participate in various meetings. During my stay at NYU, I was always delighted by her enthusiasm and willingness to help. It was PAHO/WHO who sponsored my traveling to work with Ruth, as her first Brazilian scholar, and many others were to come. Ruth seemed relentless and ever present at the laboratory, playing additional roles as our mentor, a homemaker, and a mother of three teenage children! Convinced of her scientific mission, she seemed to effortlessly overcome limits or barriers, considered insurmountable by others. Once, when she needed mosquitoes infected with *P. falciparum*, she went to Holland to fetch them personally in the only laboratory that mass-infected mosquitoes. Back to NYC Ruth carried them in a small mosquito box in her hand luggage, because she always felt the urgency and seemed to ignore the word impossible!

Ruth remained steadfast in her ideal of improving health and life worldwide and remained mindful of the scientific advances until the last months of her life (**Figure 6**). Despite her low physical mobility and being wheelchair-bound for long months during her last years, she followed publications on *P. vivax*



FIGURE 6: Ruth, a life dedicated to science. She made malaria research towards a malaria vaccine more attractive and inspired many groups to work on it.

in scientific journals with special interest. Ruth wanted very much to return to Brazil, her country at heart, where she maintained collaborators and a few old friends. She always complained of homesickness and remained closest to Judith Kloetzel, also born in Vienna from a non-religious Jewish family, who came to Brazil; her first child was named after Ruth. On my last visit to the Nussenzweigs at their NYU apartment (a must on my trips there), she complained about not fulfilling her dream and of missing Judith, who died in late 2015 in S. Paulo. We talked extensively about work and about common friends in Brazil. An old photo taken at her 50th anniversary celebration in USA shows close family members (**Figure 7**). Although Ruth's dream of coming back to Brazil was not realized, her ideas and enthusiasm remain a living flame among many of her former disciples, including me, who are deeply grateful and sorrowful.

All of Ruth and Victor's three children became scientists. Their eldest son, Michel, is a Harvard scientist and member of the Brazilian Academy of Sciences; their daughter, Sonia, who returned to Brazil decades ago, is an anthropologist and works at the School of Sociology and Politics Foundation of São Paulo; their youngest son Andre, first a physicist, now works on basic



FIGURE 7: Together with their family on the couple's 50th anniversary. Their siblings Michel, Sonia and André (arrows) are also scientists.

research in pathology and cell biology at the Cancer Institute, National Institutes of Health (NIH), USA. To the three of them, to her six grandchildren, and to Victor, my first professor of immunology at NYUMC, a great collaborator and friend, I express my sincere and deep sadness for their loss.

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