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How to understand a local enemy when fighting a global threat: a research experience from southern Colombia in the middle of a pandemic

Paula Ximena Losada Vanegas
División de Inmunología. Facultad de Salud. Universidad Surcolombiana.
Becaria del Ministerio de Ciencia, Tecnología e Innovación.
Convocatoria N° 856 de 2019: "Jóvenes Investigadores e Innovadores para el departamento del Huila".
paula.losada@udea.edu.co
Carlos Fernando Narváez Rojas
División de Inmunología. Facultad de Salud. Universidad Surcolombiana.
Doctor en Ciencias Biológicas, Pontificia Universidad Javeriana.
cfnarvaez@usco.edu.co

Abstract

Dengue virus (DENV) infection represents an important public health problem for southern Colombia. With the aim of promoting the scientific vocation in young graduate students, the local government and the Ministry of Science supported scholarships looking to contribute to the understanding of the dengue disease. Our work explores challenges in the study of DENV in a middle-income country, from the perspective of a young female physician and current Ph.D. student who developed an interest in DENV research driven by a willingness to contribute to her community. However, in the 2020, and a half, in addition to the circulation of DENV, Colombia had to face the SARS-CoV-2, and research priorities were forced to shift to target the emerging pandemic. Thus, she had to solve problems to continue with the DENV investigations, such as the low availability of reagents, the long time for their delivery, the close of the Lab for weeks, the SARS-CoV-2 infection of 80% of the Lab members, and the dead of friends and family members. The ability to adapt, the skills acquired, the accessibility of new infrastructure and specialized equipment together with the motivation to continue contributing, made it possible not only to overcome the crisis and advance DENV research but also contribute to the performance of thousands of free molecular tests for SARS-CoV-2 in the local vulnerable population. This experience contributed to the visibility of the science program in the region and the use of human and technological resources available to confront emerging infectious diseases locally.

Keywords: Ministry of Science; Scholar-internship; Young research and innovator; Dengue virus; Zika virus; COVID-19; SARS-CoV-2.

Cómo entender a un enemigo local cuando se lucha contra una amenaza global: una experiencia de investigación desde el sur de Colombia en medio de una pandemia

Resumen

Algunos agentes causantes de enfermedades graves sólo pueden sobrevivir bajo determinadas condiciones topográficas y climatológicas, pues necesitan de un vector para completar su ciclo de vida y llegar a un hospedero. Tal es el caso de la infección por virus dengue (DENV), condición que representa un importante problema de salud pública para el departamento del Huila, ya que afecta a todos los grupos poblacionales y su prevalencia es muy alta debido a las condiciones ambientales de la región, propicias para la permanente circulación del vector. En su forma más grave el DENV ocasiona daño de los órganos principales, con fuga vascular y choque, llegando a ocasionar la muerte en pocos días. Con el ánimo de contrarrestar esta problemática regional y fomentar la vocación científica en jóvenes profesionales con excelencia académica, del departamento, el Ministerio de Ciencia, Tecnología e Innovación, apoyó el desarrollo del proyecto "Efecto de la Infección DENV y ZIKV Sobre Precursores Plaquetarios", propuesta que tuvo como objetivo, a través de la recreación de un modelo experimental *in vitro*, contribuir al entendimiento de la inmunopatogénesis del dengue. Ello, en el marco de la convocatoria número 856 que, a través de la realización de becas-pasantía, posibilitó el desarrollo de proyectos que respondieran a problemáticas locales. Cabe resaltar que durante el periodo de desarrollo de la beca el mundo se detuvo por la aparición de un virus respiratorio denominado SARS-Cov2, altamente infeccioso y mortal, que se convirtió en pocos meses en pandemia. Como consecuencia todos los países tuvieron que implementar medidas sanitarias con restricciones extremas de aislamiento, lo que representó un reto importante para continuar con las investigaciones y con la vida misma. Sin embargo, la capacidad de adaptación, las habilidades adquiridas, la accesibilidad a infraestructura idónea y equipos especializados, junto con la motivación por continuar contribuyendo a la ciencia hicieron que, no sólo se pudiera superar la crisis y avanzar en el proyecto, también que desde la división se aportara con la realización de pruebas diagnósticas para SARS-CoV2. Además del aporte académico, resultado de la investigación, dicha experiencia contribuyó a dar visibilidad al programa Jóvenes Investigadores e Innovadores para el departamento del Huila, al aprovechamiento de los recursos humanos y tecnológicos con los que cuenta la División de Inmunología de la Facultad de Salud y a fortalecer las capacidades investigativas de una joven médica para dar continuidad a su formación de nivel doctoral.

Palabras Clave: Ministerio de Ciencia, beca-pasantía, Joven Investigador e Innovador, virus dengue, virus Zika, COVID-19; SARS-CoV-2

Context

The geographical location of Colombia and the topography of Huila's department make the region a favorable place for the growth and development of the Aedes aegypti mosquito, vector of Dengue (DENV) and Zika (ZIKV) viruses, added to the tropical climate during through the year allows the constant circulation of the vector and therefore the risk of permanent infection in the population. In fact, DENV infection is considered a public health problem, since according to epidemiological behavior, at least 500 cases per 100,000 inhabitants are estimated, of which, on average, 169 cases become serious or lead to death without discriminating race, gender, age or social condition (Castrillón et al., 2015).

Mentioning this pathology as a possible differential diagnosis of any febrile syndrome is common in the medical consultation but it transmits fear to patients in the region, who, through their own experience or through close people, have known how devastating the infection can be. The spectrum of symptoms is very wide, from fever and rash, arthralgia, headache, and retro-ocular pain, through abdominal pain, and spontaneous bleeding, to severe bleeding, seizures, organ damage, and finally death in an important number of cases (Guzman & Harris, 2015). Within the clinical approach of any febrile condition when flavivirus infection is suspected, a high relevance is given to the count of a type of cells important in the control of bleeding, such as platelets (Musso et al., 2019). In the case of DENV infection, a low platelet count is expected after the fifth day of illness (Halstead, 2007). The role that platelets would have and the effect that DENV has on this cell type are not well known.

Since the Zika virus (ZIKV) epidemic between 2015–2016 in DENV endemic regions, many groups have investigated whether ZIKV infection in individuals with prior immunity to dengue could worsen the disease, similar to the effects of secondary heterotypic infection by DENV as a mechanism of antibody-dependent amplification (ADE) (Katzelnick et al., 2020) . One characteristic that differentiates them is that these flavivirus have different cellular tropisms. Human and mouse models show that cells of the monocyte/macrophage lineage and dendritic cells are the major targets of infection for DENV in organs such as skin, liver, lymphoid, and non-lymphoid tissue, while studies in human samples and cell cultures, primate and mouse models show that ZIKV infection is pantropic (Begum et al., 2019). Therefore, being able to perform the experimental model comparing platelet infection by these two viruses is so exciting.

Understanding these two arboviruses, especially the disease caused by DENV, is a priority need in the region to establish possible therapeutic alternatives. For this, thanks to the call 856 of 2019 offered by the Ministry of Science, Technology, and Innovation, the Immunology Division had the involvement of a young researcher and innovator (YRI) for the Department of Huila thanks that the Universidad Surcolombiana is part of the National System of Science, Technology, and Innovation.

During the one-year fellowship-internship as YRI at the Infection and Immunity Laboratory (I&I Lab), it was possible to advance in the project EFFECT OF THE INFECTION OF DENV AND ZIKV VIRUSES ON PLATELET PRECURSORS, an in vitro study that described the effect that produced by DENV and ZIKV on human platelet precursors in an attempt to understand the immunopathology of the disease and explain thrombocytopenia seen in DENV-infected patients.

To develop the project, it required the use of various techniques and methodologies to reproduce the *in vitro* infection model with platelets. It is known that obtaining the progenitors requires invasive methods such as bone marrow aspiration, a highly invasive and painful medical procedure that place patients at risk. That is why the commercial MEG-01 cell line was used, a megakaryocyte lineage isolated from a patient with chronic myeloid leukemia, thanks to a collaboration with the laboratory of Lee Gehrke, Massachusetts Institute of

Technology MIT, USA. Additionally, working with this cell line requires the availability of ultra-low freezers or liquid nitrogen storage tanks for the cryopreservation of the cells for a long time for later use.

Previous training was required for the development of the necessary techniques, with the performance of previous tests with a different type of cell known as VERO 76, which is widely used by the scientific community for *in vitro* infection models, especially for DENV. This cell line is commercially known to be obtained from the kidney of a species of African green monkey, called *Cercopithecus aethiops.*

Starting to cultivate the MEG-01 progenitors cell line requires basic knowledge and to be careful in maintaining the optimal conditions necessary for the survival of the cells, which by the way, are very fragile to changes in temperature, pH, and humidity, without mention the measurements of necessary good practices of asepsis to avoid possible contamination by bacteria. One of the greatest difficulties was to guarantee the conservation of the cells, since the liquid nitrogen necessary for cryopreservation evaporates quickly due to its physical-chemical properties and its acquisition is guaranteed thanks to the shipment from the city of Bogotá. In addition, once the culture has started, time is required almost permanently to avoid the cell, since it is required to maintain the necessary nutrients present in a solution called culture medium which must be renewed according to cell growth.

In order to reproduce thrombopoiesis *in vitro*, it was necessary to start the progenitor´s development, for this, it is common to use phorbol-12-myristate-13-acetate (PMA), an ester that induces the differentiation of the progenitors to mature platelets (Banerjee et al., 2020). Given that, the objective was to evaluate the effect of the infection during the different stages of the thrombocytes until they became platelets; the next step was to infect the progenitors at three different times, before, during, and after stimulation with PMA. Then, the viruses were inoculated into the culture medium with the growing cells and after hours of transmission, the characterization of the infected platelet progenitors was carried out.

Thanks to the availability of flow cytometry technology, a technique useful for the evaluation and characterization of cellular parameters at the individual level, it was possible to evaluate markers of the cell line under study that have been previously described in the literature as proteins present on the cell surface that indicated activation and/or differentiation of cells at different times of infection (Banerjee et al., 2020). The last one was determined intracellularly with the use of monoclonal antibodies directed specifically against each of the viruses and subsequently, a secondary antibody coupled to a fluorescent marker to also be detected by flow cytometry.

Once all the information obtained was analyzed, it could be concluded that both flavivirus can infect human platelet precursors (although differentially) and that DENV but not ZIKV affects cell viability. This suggests that infection by these two Flaviviruses affects thrombopoiesis at different intensities (Losada PX et al. *in preparation*. 2021).

In March of 2020, the SARS-CoV-2 arrived in Colombia. As a result of the health crisis due to the new Coronavirus pandemic and the social isolation measures that were adopted throughout the national territory, all kinds of activities were suspended. This implied that the academic, administrative, and research activities were completely stopped for more than 16 weeks, which forced me to rethink the way to carry out the plan established for the year of the scholarship. The adoption of online team meetings allowed for maintaining internal communication and continuing with the training and development of research from virtuality, focused particularly on the update of relevant new references and writing of preliminary results.

It is unimaginable to be able to continue an investigation of this magnitude with so many particular situations under virtuality. Due to the impossibility of going to the laboratory and the consequent delay in the

development of experimental activities, an important emotional commitment was added to the situation. People closer such as family, friends, acquaintances, and medical colleagues get infected by SARS-CoV-2, some of whom lost their lives due to direct exposure to infected people trying to fight the virus in the front line. Also, 80% of the lab members got symptomatic infections, and several of them required oxygen support and steroid therapy. However, this led to the fact that, despite not being able to carry out a stage of mourning, it was necessary to assimilate everything that had been experienced reasonably and to start the academic and research life again and with greater force, especially in memory of those who lost the battle. Now, jointly with the lab team survivors of the infection value the role of science not only for the development of the regions but also as the best tool to guarantee the survival of the human species.

Great ideas often come from a combination of knowledge and experience in adverse situations. The pandemic recalled the importance of ensuring the availability of scientific information and easy access to the latest discoveries in any area. Thanks to this, many online platforms released documents that were not previously freely accessible. During the time of confinement, one way to adapt and continue with the research process was to improve the skills for searching manuscripts. Among all the possible types of text that are regularly published, those that are the product of original research are vital to know the methodologies previously used and to have a point of reference for the development of one's own ideas. Thus, the literature review played a crucial role in planning the experimental model and preliminary parts of the body text.

In the context of the pandemic, while the research with arboviruses and MEG-01 was developed, the Immunology Division also had to face a new challenge and this time it was to contribute to the department of Huila, not only with the research of DENV and ZIKV, but also with the performance of molecular diagnostic tests for COVID-19. Thanks to the low regional experience in the use of molecular technical for diagnosis of human viral diseases, it was possible to establish an agreement with the departmental government that allowed a collaboration with the public health laboratory that resulted in the realization of thousands of free molecular tests for the diagnosis of SARS-CoV-2 with the aim of increasing the diagnostic capacity of the department. Specifically, this led to the performance of the real-time reverse transcription-polymerase chain reaction (RT-qPCR) test, which, although not directly related to the linking project, largely complemented the scientific training of the YRI, one of the main objectives of the Young Researchers program. Additionally, the Division of Immunology won a government grant for the strengthening of the investigative and diagnostic capacity of viral diseases. This achievement allowed the improvement of the infrastructure of the Infection and Immunity Laboratory (I&I Lab), which was equipped with modern and specialized equipment in addition to the training and updating of all the team such as laboratory assistants, students in special internships, bacteriologists, the young intern, who had frequent talks on topics related to issues such as Lab quality processes.

This reorganization, which had to be implemented as a consequence of the pandemic, also involved weekly meetings of the entire staff, necessary to analyze the team's performance and also to discuss diagnostic tests for viruses from an academic perspective. During these meetings, there was significant feedback to correct and/or strengthen concepts throughout the team.

Actors involved

Considering that dengue is a common concern in Colombia and the department of Huila, several local actors were committed to generating new knowledge and strengthening research in the region. The type of contribution made by each actor from an academic, scientific, and administrative point of view is briefly described below:

- *Young Researcher and Innovator Position:* The role of a YRI within the development of the project was the realization of experimental techniques such as cell culture, immunostaining, flow cytometry, and light microscopy, among others, which were required to evaluate the effects of viruses on cells.

- *Immunology Division at Universidad Surcolombiana:* Provided the technical and scientific staff who contributed with their experience and knowledge to the development of the proposal and a solution to problems presented along the way in addition to offering all the technological tools with the most specialized equipment in the region, as well as the use of modern infrastructure, got during pandemic.

- *Government of the Department of Huila:* Supported the initiative of the Young Researchers and Innovators program for the Department of Huila, a pioneer of its kind in Colombia, and provided the necessary financial resources by providing a percentage of the department's royalties to finance the scholarship-internship.

- *Ministry of Science, Technology, and Innovation:* Together with the government, it generated the call N° 856 that made it possible to link the YRI to the Immunology Division in order to promote the scientific vocation of young professionals in Huila, southern Colombia.

Impact of experience

The scholarship internship represented a great opportunity to keep scientific activity alive in the region and promoted development not only at the individual level by linking Young Researchers to research group projects, but also, by placing science as one of the pillars for the development of the department.

Without a doubt, the scientific contribution of this study goes beyond having DENV infect the human platelet cell line throughout its development and maturation since this research contributes significantly to the generation of new knowledge and a better understanding of the immunopathology of infection. Once they have been functionally and phenotypically characterized in the different moments of thrombopoiesis, it is possible to think about the selection of possible predictive markers of severity or evolution of the disease, which leads to other questions that will be resolved with future research. In addition, all these findings can guide clinicians to carry out timely interventions in patients just before the onset of the critical phase where a decrease in the number of platelets in peripheral blood is expected, as a consequence the patient resulted in shock and ultimately death.

The best way to guarantee the progress of the department was focusing economic resources on research and taking advantage of the human talent that has been formed in the region such as young professional researchers who are Huilenes graduated from local institutions.

It is interesting to see how the role of women in science has become increasingly relevant, without displacing the male gender, and much more challenging to maintain the motivation to do science when the resources allocated to it are so limited in the nation. This was an opportunity to continue doing basic science although it is rare in our local society, and much stranger for a woman of the medical profession to decide to continue this path, as in the case of the YRI involved in this project.

The Infection and Immunity laboratory has been a place of passage for many medical students who have been linked to the Semillero de Formación en Infección e Inmunidad (SFI&I) and now, every member can work with modern and specialized equipment and a good infrastructure. This is a unique advantage that the Faculty of Health and the Universidad Surcolombiana have over other academic institutions in the region.

- Strengthening of research groups.

The experience provides the linkage of young researchers to seedbeds and research groups which guarantees a strengthening and in turn reciprocal training between young researchers, students, and directors, since it contributes with innovative and recent ideas both for the organization of the laboratory and development of new ideas to carry out more innovation projects.

- Strengthening of higher education institutions in scientific contributions

Experiences like this have promoted and inspired research in students from undergraduate to later guarantee their connection as a young researcher, an increase in scientific production is also guaranteed and, incidentally, gives a better academic position to university.

Learning and lessons learned

The development of the project allowed me, from the investigative experience, to know the importance of studying one of the main infectious agents in the region, such as DENV.

In the same way, the research life of young people with a scientific vocation was encouraged and strengthened, with the support and direct link to the research group under the figure of YRI.

This model program motivates the students to guide their lives toward the scientific live from the Without a doubt, the scientific contribution. It is crucial to guarantee the continuous training of students with critical thinking and research skills from the first levels of schooling to obtaining the highest level of academic and research training, always having the objective that throughout this process contribute to the construction of a better scientific society that is capable of improving living conditions based on their contributions.

As a big final message, the pandemic has left important lessons, but the most relevant was to remind the world about the importance of science to work for human life and the importance of the adequate distribution of economic resources and humans available for the Department of Huila.

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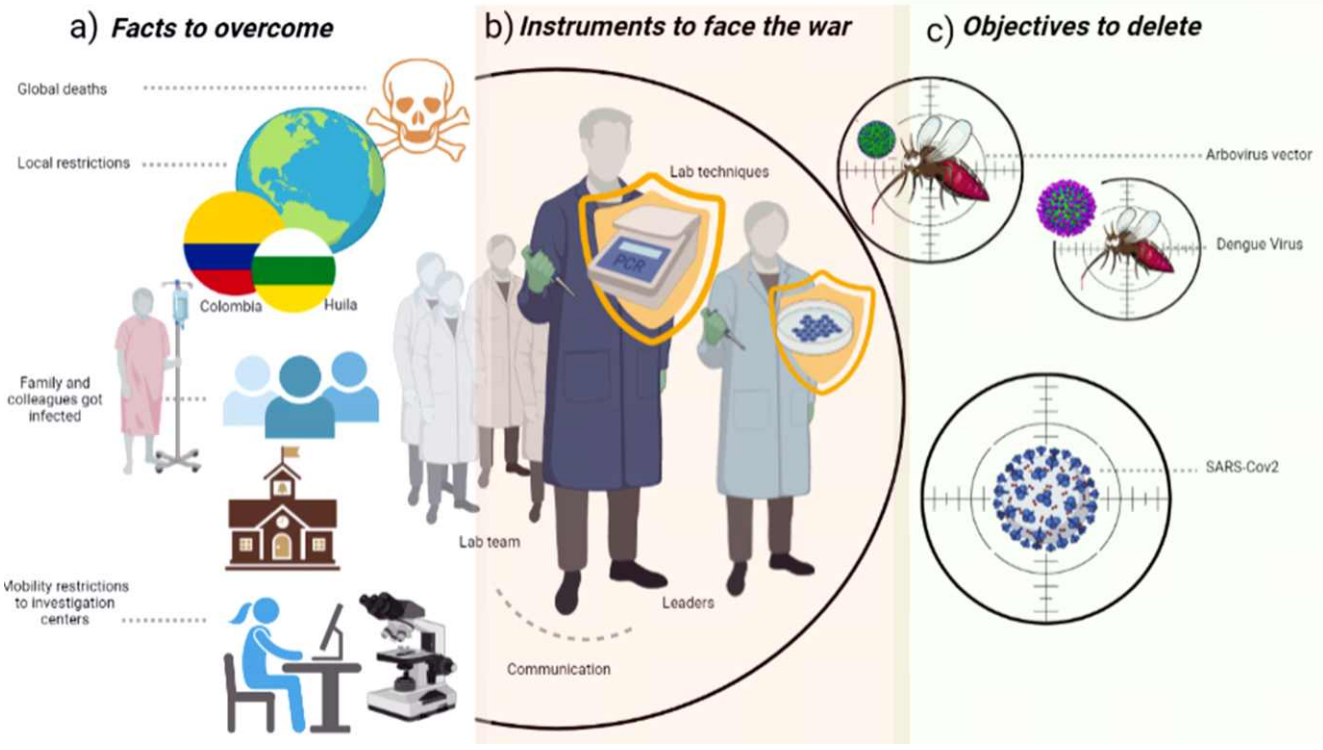
Figure 1

Challenges of DENV investigation in the middle of SARS-CoV2 pandemic.



There were several challenges that had to be overcome to continue the research in the middle of the global crisis caused by the pandemic. a) There were some facts to overcome: Among those out the emotional component due to the impact on the health and loss of life of family, friends, acquaintances, and colleagues, also due the mobility restrictions most of the work had to be virtual. b) Instruments to face the war: The confinement took 16 weeks and delayed many advances in the project but that generated greater communication between the lab team. Thanks to this, it was possible to make use of the physical resources available in the Infection and Immunity Laboratory, and under special perks, the whole team could be mobilized to continue the investigation. c) Finally, the laboratory objectives changed, and we could face not only DENV but also help in the diagnosis of SARS-CoV-2 locally.