Leishmaniasis: an overlooked parasitic disease

Molecular geneticist Dr Ikram Guizani leads a Tunisian team working to find a new treatment for leishmaniasis. She discusses the weaknesses of existing drugs, and explains how collaborations with similar institutes across the globe helped highlight a new molecular target.

How did you become involved in research into Leishmania, the parasite responsible for leishmaniasis?

Upon returning to Tunisia in 1989, after completing my PhD in Molecular Genetics in France, I was appointed at the Institut Pasteur de Tunis. I joined a multidisciplinary group of researchers to study leishmaniases, major public health problems in Tunisia. Thanks to my background, I was able to pioneer molecular parasitology studies, and develop and apply DNA probes for the genetic characterisation of Leishmania parasites. I went on to train students and establish my own team, while further developing my research and diversifying molecular biology approaches to study these parasites.

What are the challenges in combating leishmaniases?

Leishmaniases are caused by multiple Leishmania species and involve diverse transmission cycles. The control of these diseases depends on diagnosis and treatment as well as prevention measures, such as vector or reservoir control. Adapting effective measures and scaling up solutions constitute real challenges for many reasons, including disease distribution patterns, transmission cycle diversity and costs. In addition, there are very few treatment options available. Drugs are costly and may have severe side effects. Moreover, increased drug resistance is being reported for the mainstay therapy, pentavalent antimonials. As a result, miltefosine – which has demonstrated activity against various parasite species – is being used in the Indian subcontinent; however, relapse cases are increasingly being reported for this drug. In addition, different species and strains have varying sensitivities to the drugs in use; therefore, novel diagnostics, drugs and vaccines are important research priorities.

Why did you choose to test Leishmania eukaryotic initiation factor (LeIF) as an anti-infective protein?

This protein has been identified as a natural Th1 adjuvant with the ability to induce cytokine expression, including interleukin-12 (IL12). Dr Mourad Barhoumi, a postdoc in my laboratory who completed his PhD on a project studying LeIF, had the idea to test it as an anti-infective protein. He was able to develop these experiments while visiting the cellular immunology laboratory of Dr Eleni Dotsika at the Hellenic Pasteur Institute in Greece. Dotsika has robust experience in immunology and Leishmania infection models, and she was analysing immune responses to LeIF peptides at the time. As a result, a collaboration was established.

A LeIF recombinant protein vaccine has been shown to be protective in mouse models. Do you see it having potential as a future leishmaniases treatment?

Our work suggests that LeIF may constitute an active biomolecule that promotes resistance of the macrophage to infection, thereby reducing lesion growth and tissue invasion. With Dotsika’s help, we are currently developing different models of infection to evaluate LeIF’s therapeutic potential. I hope our findings will promote its clinical application.

Have collaborations – such as those with your colleagues in Greece – furthered your work on Leishmania?

Collaboration is important to advance our research because it brings together complementary expertise and allows access to infrastructure. It is also an important mechanism for knowledge transfer and building research capacity. We have excellent collaborations in Tunisia and elsewhere, particularly with teams within the Institut Pasteur International Network and at the Institut Pasteur: An important aspect of this collaboration is the training of young researchers while addressing our common objectives. All our collaborations are beneficial to our activities and result in high-quality results and publications. The Transversal Research Programme of the Institut Pasteur has been pivotal in supporting our ongoing activities on LeIF, which enabled the establishment of fruitful collaboration with teams at the Institut Pasteur, the Hellenic Pasteur Institute and the Institute of Physical and Chemical Biology in Paris, France. We are also grateful for the support of the Ministry of Higher Education and Scientific Research and Technology, Tunisia, provided through research programme contracts, and of the Special Programme for Research and Training in Tropical Diseases (TDR), Switzerland, during our initial project.

What roles have you assumed in the World Health Organization (WHO) to promote the cause of leishmaniasis?

I have contributed to WHO’s TDR through steering committees, scientific meetings, projects and networks. I have also contributed to activities developed by the WHO Regional Office for the Eastern Mediterranean. My role, as an expert from a disease endemic country, is to promote research and training for infectious diseases linked to poverty, including leishmaniases, and facilitate and foster leadership, empowerment, innovation and translation for public health impact in disease endemic countries. My role is also to advocate for access to healthcare, scientific knowledge and technologies as basic human rights and requisites for societal development.
Parasitic protein or disease cure?

Researchers at the Institut Pasteur de Tunis and Hellenic Pasteur Institute have identified a molecule that inhibits the growth of the parasite behind visceral leishmaniasis, a neglected tropical disease that can be fatal if left untreated.

**LEISHMANIASES, A GROUP** of clinically diverse diseases, are caused by parasites belonging to the genus *Leishmania*. Transmitted by sandflies, the disease has three main forms: cutaneous, which presents with skin ulcers; mucocutaneous, which presents with mutilating ulcers of the mouth and nose; and visceral, which results in fever, low red blood cell count, and an enlarged spleen and liver. If left untreated, visceral infections can be fatal, and even those fortunate enough to recover from the cutaneous forms are left with permanent, disfiguring scars.

These diseases are a major public health problem, affecting millions of people across the globe. Considering its burden expressed in disability-adjusted life years, each of which represents one lost year of healthy life, leishmaniasis is ranked third among all parasitic diseases. Because it primarily affects populations with limited resources and access to healthcare, the disease is insufficiently controlled. However, there is not just an issue of access; treatments themselves are also severely lacking. Available drugs require long treatment courses and must be delivered in a hospital. As well as the method of delivery, the drugs are expensive and often have toxic side effects. Add to this the fact that resistance is beginning to emerge, and it is clear that alternatives are needed.

An important figure in the fight against leishmaniasis is Dr Ikram Guizani. She leads the Laboratory of Molecular Epidemiology and Experimental Pathology at the Institut Pasteur de Tunis, where she is conducting research to find new treatments and methods of diagnosis for leishmaniasis.

**PARASITE SEQUENCING**

Diagnosing the precise form of leishmaniasis a patient is suffering from is important. There are over 20 species of *Leishmania* parasites, and each reacts differently to different drugs. Therefore, knowing the parasite species that caused the disease can ensure the most appropriate treatment is administered and vastly improve patient outcome.

Guizani’s team is thus working to create new diagnostic kits. Using her background in molecular genetics, and in partnership with the Institute’s Clinical Investigation Center and Skuldtech, a biotechnology company in France, she is developing DNA assays to identify the parasitic species causing an individual’s disease. The lab is also using genomic approaches, including *Leishmania* next-generation sequencing, to identify genetic markers of the disease, particularly the most dangerous visceral form. In time, the researchers hope to translate their findings into novel diagnostic approaches. “The steps we are taking now with genome analyses should logically result in the identification of disease determinants,” Guizani expands.

**TARGET IDENTIFICATION**

This work could also identify new targets, such as molecules, for intervention. Indeed, developing new therapeutics using natural products or rational approaches is the major focus of the lab’s efforts. To design new drugs rationally, the scientists are focusing on essential or unique pathways in pathogenesis, aiming to find a molecule that is significantly different from host proteins, has druggable binding pockets, is essential for the parasite’s survival and is easily assayable.

Through their efforts, the team identified *Leishmania* eukaryotic Initiation Factor 4A (LeIF), a TH1 type immune modulator. This protein is expressed in the amastigotes, the most infectious form of the parasite and the most important stage to target with drugs. It is also well conserved across *Leishmania* species, including *L. donovani* – the second most prevalent species and the most dangerous form of the parasite, as it is fatal to humans. After recombinant expression, biochemical characterisation of this promising protein and identification of its unique characteristics, the team began experiments to elucidate its function during infection.

**PARADOXICAL PROTEIN**

When the body is infected with *Leishmania*, it mounts an immune response. Helper T-cells, especially Th1, produce signalling proteins called chemokines and cytokines. These chemicals activate white blood cells called macrophages, which protect against *Leishmania*. However, when the immune system fails to react sufficiently, these cells start to act as the host cell for the parasite.

Leishmaniasis develops when the parasite overcomes or disrupts the immune response, or evades it completely, in order to establish and maintain infection. *Leishmania* can modulate the immune response through the alteration of chemokine and cytokine
Leishmaniasis is likely to become more prevalent in the future, as climate change encourages its spread, and urbanisation, migration, occupation and conflict increase transmission of the disease.

**Statistics from: World Health Organization (WHO)**

- **12 million** people are affected with some form of leishmaniasis in 98 countries
- **2 million** new cases of leishmaniasis are annually recorded including about **300,000** cases of visceral leishmaniasis

The annual death toll is between **20,000** and **50,000**. The only parasitic disease that surpasses these figures is malaria.

Over **90%** of new visceral leishmaniasis cases – the most deadly form of the disease – occur in Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan.

More than **two-thirds** of new cutaneous leishmaniasis cases – the most common form of the disease – occur in Afghanistan, Algeria, Brazil, Colombia, Iran and the Syrian Arab Republic.

Almost **90%** of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil and Peru.

**Unanswered Questions**

The findings Guizani and her collaborators have made to date have been astounding, but how LeIF prevents Leishmania infection is not yet completely understood. Many important questions remain; notably if LeIF has the ability to induce resistance to other Leishmania species, and how its actions are exerted and balanced during infection. Guizani reveals: To close these knowledge gaps, she is currently investigating the model of infection in another Leishmania species: “We have evidence that LeIF induces resistance to infection with another species, and that it activates intracellular kinases that are important for survival,” she elucidates.

Although Guizani does not yet know exactly how LeIF inhibits the infection, the important aspect is that it does. In fact, a trisaccharide recombinant protein vaccine that includes the NH terminal part of LeIF has already been developed as a molecule and a strong clinical candidate.

**Inhibition of Intracellular Parasite Growth by LeIF**

**Objective**

To explore the potential of Leishmania infantum eukaryotic initiation factor (LeIF), an exosomal protein, as a novel anti-infective therapeutic molecule and drug target to treat leishmaniasis.

**Key Collaborators**

- Dr Amel Garnauoi, Dr Mourad Barhoumi, Dr Khadija Ben Khadir Essafi, Dr Habib Karoui, Ms Emna Harigua, Ms Yosser Zina Abdelkrim, Ms Imen Bassoumi, Ms Ons Zakraoui, Institut Pasteur de Tunis, Tunisia
- Dr Eleti Dotsika, Dr Olga Koutsoni, Dr John Kyriazis, Hellenic Pasteur Institute, Greece
- Dr Michael Nitges, Dr Guillaume Bouvier, Dr Arnaud Blondel, Dr Hélène Munier-Lehmann, Dr Gerald Spaeth, Institut Pasteur, France
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**Partners**

- Institut Pasteur de Tunis, Tunisia
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**Ikram Guizani** earned her Master’s degree in Molecular and Cellular Genetics from the University of Paris XI in 1982, completed her PhD at the University of Nice in 1986 and finished a State Doctorate at the Tunis El Manar University in 2002. She is currently Principal Biologist and Head of the Laboratory of Molecular Epidemiology and Experimental Pathology (LR 11 IPT 04) at the Institut Pasteur de Tunis.