Leishmaniasis in the laboratory

Ketty Soteriadou, PhD, details the work her laboratory has conducted into the pharmacology of leishmaniasis, a debilitating parasitic disease that is second only to malaria in deadliness.

Could you introduce yourself and your professional background?

I am currently the Emeritus Research Director and Head of the Molecular Parasitology Laboratory (MPL) at the Hellenic Pasteur Institute (HPI). In 1972, I graduated from the Pankiprion Gimnasion Arrenon Kikkou in Nicosia-Cyprus, and in 1979 I obtained degrees in Biochemistry (Université Paris VI) and Human Biology (Université Paris XI). At that time I was appointed as a research assistant in the Virology Department at the HPI. Subsequently I moved to the Biochemistry Department, and in 1983 I obtained my PhD in Microbiology and Virology from the Université Paris Diderot, before becoming a research associate and group leader of the Leishmania project in 1988. I was appointed Head of the MPL in 1998 and promoted to Research Director in 2006.

Leishmania provides a good model for system biology studies involving evolution. What opportunities does this represent for researchers?

From an evolutionary viewpoint, one can exploit the fact that the dynamics of Leishmania transmission involves two other players: the sand fly vector and the mammalian host. Comparative study of molecular markers based on these systems may shed light on crucial evolutionary questions. Furthermore, by using a systems biology approach, comparative genomics from sequenced genomes, and proteomics, one may address critical evolutionary questions that are still being debated (for example, the origin of introns and their role in eukaryogenesis, and the evolution of the spliceosome and the mitochondrial endosymbiont, as well as important pathways such as ubiquitin signalling and nucleo-cytoplasmic transport).

Why has leishmaniasis been relatively neglected in comparison to other tropical diseases?

Leishmaniasis, a poverty-related disease that affects the poorest of the poor, is the second largest parasitic killer in the world; only malaria is more deadly. However, pharmaceutical interest in the development of new effective, low-cost drugs is extremely low, and funds from international organisations, the EC and even national funding agencies are very limited. Stakeholders underestimate the disease’s impact on public health. There is a need to increase awareness of its significance within communities at international, national and local levels.

The MPL is currently conducting research on aspirin resistance in cardiovascular disease. Could you provide an insight into some of the work you are doing in this area?

Aspirin resistance (or ‘unresponsiveness’) is believed to be multi-factorial. Measuring the response to aspirin is difficult and there is no one assay that can be recommended for routine clinical practice. Notably, dual antiplatelet therapy with aspirin and clopidogrel is currently the gold standard therapy for patients with cardiovascular disease. In this context, we aim to correlate this phenomenon with clinical, biochemical and genetic factors. In particular, we seek to delineate the relationship between aspirin and clopidogrel resistance and genetics using a genotyping strategy. We study the polymorphism of genes directly involved in the drug’s mechanism of action as well as genes in other pathways, such as metabolic pathways that may modulate this response.

Is the work being conducted within your laboratory contributing towards personalised medicine?

Our research aims to correlate aspirin and clopidogrel resistance, or treatment failure with genetic predispositions, falls within the field of pharmacogenomics. This area of intensive research aims to measure the influence of genotype on pharmacology, leading to the development of personalised treatment programmes and individualised drug selection for improved safety, efficacy, cost and sustainability.

In the case of leishmaniasis, the characterisation of both parasite and host biomarkers that determine the passage from infection to disease may affect the choice of appropriate treatments.

Does the MPL work with other laboratories or organisations on the creation of leishmaniasis control and management strategies?

A multidisciplinary approach is a prerequisite for the success of our work. Members of the MPL collaborate with the National Reference Center for Leishmaniasis, another branch of the HPI. The Center, headed by my colleague Eleni Dotsika, is funded by the Ministry of Health and reports to public health authorities. Within the HPI, we also work with members of the Laboratories of Medical Microbiology, Cellular Immunology and Molecular Virology, whilst externally we are currently collaborating with laboratories in Greece, France, Cyprus, Germany, Turkey and Brazil.

Who has helped you get where you are today?

Above all, I would like to thank my parents for giving me values in life and for their deep love and encouragement. I would also like to express my deep thanks to my family, Kyprianos Nicolaides, Myrna and Socratis, and my beloved brother. I would like to express my gratitude to my teachers and friends in high school and in life.

I am also grateful to the HPI and to my mentors Socrates Tzartos (HPI), Genevieve Milon (Institut Pasteur in Paris) and KP Chang (Chicago), as well as to all my collaborators – especially HPI researchers Christos Haralambous and Despina Smirlis.
Pondering parasites

The Molecular Parasitology Laboratory at the Hellenic Pasteur Institute has been conducting research into a group of parasitic diseases known as leishmaniasis, with a view to developing novel treatments and determining biomarkers.

LEISHMANIASIS IS A disease caused by protozoan pathogens of the Leishmania genus. It is transmitted by the female sand fly and can affect both humans and animals, with the sand fly acquiring the parasite when it sucks blood from an infected host.

There are different forms of leishmaniasis, which are of varying severity and can express a wide range of clinical symptoms. These include cutaneous leishmaniasis (affecting the skin), mucocutaneous leishmaniasis (affecting the mucous membranes) and visceral leishmaniasis (affecting the internal organs). Visceral leishmaniasis is the most aggressive form of the disease, and can be life-threatening if left untreated.

THE RISE OF LEISHMANIASIS

Cases of leishmaniasis are rising around the world. This is due to a number of factors, including climate change, population movement, conflicts and malnutrition in highly endemic areas, and rising levels of drug resistance. These are exacerbated by an inadequate supply of drugs, lack of an effective vaccine and insufficient control measures.

According to the World Health Organization (WHO), leishmaniasis is endemic in 88 countries, and affects approximately 12 million people worldwide, with this figure set to increase by a further 1-2 million cases per year. Leishmaniasis is a serious public health issue, with debilitating socioeconomic impacts within developing but also developed countries. Visceral leishmaniasis is the most serious health issue, with cases of leishmaniasis control.

The Molecular Parasitology Laboratory (MPL) at the Hellenic Pasteur Institute in Athens, Greece, is committed to researching and developing treatments for leishmaniasis. For over 30 years, the main focus of the laboratory has been on the Leishmania parasites that cause the different forms of leishmaniasis. The scientists employ a combination of human, insect and animal biotechnological methods to gain a better picture of parasite-host interactions.

The MPL also examines other trypanosomatids, such as Trypanosoma brucei, as well as cardiovascular diseases. As a lower eukaryotic organism (an organism whose cells contain a nucleus and other structures surrounded by a membrane, as opposed to prokaryotes like bacteria), Leishmania serves as a model for systems biology studies that aim to shed light on processes such as evolution and adaptation.

ADDRESSING PROBLEMS

At present, a diagnosis of leishmaniasis is acquired through the detection of parasite DNA in tissue or human blood — a serological diagnosis may include an immunofluorescence antibody test and immunochromatography. However, such testing is not always entirely accurate. Similarly, although the development of new drugs has increased significantly over the past decade, each new drug presents its own set of problems relating to toxicity, cost and tolerance. Therefore, the MPL is working to improve upon current diagnostic tools and therapeutic strategies. It is doing so via the utilisation of polymerase chain reaction (PCR) and real-time PCR in combination with serology for sensitive diagnosis and follow-up, as well as targeted treatment.

An additional problem associated with leishmaniasis is that domestic dogs act as reservoir hosts for Leishmania; canine leishmaniasis is endemic in over 70 countries worldwide, including several in the Mediterranean and south-eastern Europe. “Canine leishmaniasis management is challenging,” explains Ketty Soteriadou, PhD, Emeritus Research Director and Head of the MPL. “This is due to the complexity of clinical manifestations and the lack of 100 per cent sensitive and specific diagnostic tools.” Accordingly, MPL researchers have been investigating the use of serology and real-time PCR, in combination with novel drug treatments and sand fly repellents, to reduce cases of canine leishmaniasis and prevent further transfer of the disease to human hosts.

TREATMENT OF CARDIOVASCULAR DISEASES

MPL scientists are also conducting research into the influence of clinical, biochemical and genetic factors on unresponsiveness to treatments for...
Cardiovascular disease. They are particularly interested in the relationship between aspirin resistance and genetic predispositions. Though this venture may at first appear to differ from research into parasitic diseases, the two areas are in fact intimately connected: an insight into the mechanisms that affect resistance to treatments may influence the choice of appropriate treatments for parasitic diseases, as the underlying mechanisms have been shown to exhibit similar characteristics.

NEW STRAINS

So far, the researchers at the MPL have made a number of important findings. In 2012, for example, new discoveries helped to shed light on the epidemiology of leishmaniasis in south-eastern Europe. Using multilocus microsatellite typing technology (MLMT), a collaborative group of researchers discovered a new group of *Leishmania* strains originating from Turkey and Cyprus and able to cause both cutaneous and visceral leishmaniasis. This population has been demonstrated to be genetically different from the populations present in other European countries. The results of this discovery suggest that the epidemiology of leishmaniasis is more complicated than originally thought, and also indicate that previous typing methods may have been ineffective, further supporting the call for a revision of *Leishmania* taxonomy.

MPL scientists were also behind the discovery that parasites that overexpress the *Leishmania* histone H1 are not infectious – a finding that could play a crucial role in the development of a vaccine.

FURTHER DEVELOPMENTS

MPL researchers will continue to work on elucidating trypanosomatid biology and the roles of parasite molecules in parasite growth and infectivity/virulence – with a focus on specific parasite molecules, such as H1 and glycogen synthase kinase (GSK-3) – and will continue to study the epidemiology of leishmaniasis in the Mediterranean and south-eastern Europe. Ultimately, they hope to advance understanding of *Leishmania* genetics to the point of developing novel diagnostic tools and treatments. “Our general objective is to contribute to the establishment of a surveillance system in these regions and to promote trans-border control strategies,” Soteriadou highlights.

INTELLIGENCE

MOLECULAR PARASITOLOGY LABORATORY

OBJECTIVES

To determine the pathogen factors that play a key role in parasite cell and life cycles as well as infectivity/pathogenicity; identify and study putative drug targets in trypanosomatidae, and to determine new molecules with antiparasitic activity; and develop molecular methods for the diagnosis and typing of *Leishmania* species, as well as conduct population genetic analysis of strains isolated in south-eastern Europe.

CURRENT KEY COLLABORATORS

Greece: Medical Microbiology, Cellular Immunology and Molecular Virology Laboratories, Hellenic Pasteur Institute; Assistant Professor Maria Antoniou, University of Crete; Professor Leonidas Sklutsinos, National & Kapodistrian University of Athens; George Panayotou, PhD, Biomedical Sciences Research Center “Alexander Fleming”; Professors Alexandros Tselepis and Vassilis Tsiliris, University of Ioanna

Turkey: Professors Seray Ozensoy Toz and Yusuf Özbel, Ege University Medical School

Cyprus: Vasiliki Christodoulou, PhD, DVM, Veterinary Services of Cyprus

France: Gerald Spaeth, PhD, Institut Pasteur; Laboratoire de Parasitologie Centre National de Référence des Leishmania

Germany: Gabriele Schönian, PhD, Charité University Medicine Berlin

Brazil: Milena B P Soares, PhD, Centro de Pesquisas Goncalo Moniz, Fundacao Oswaldo Cruz

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KETTY SOTERIADOU, PhD, has published over 60 articles in peer-reviewed journals, and supervised 14 PhD candidates and five postdoctoral fellows.

THE MOLECULAR PARASITOLOGY LABORATORY

The laboratory concentrates its efforts upon three main research goals:

- The determination of pathogen factors that affect parasite cell and life cycles as well as infectivity/pathogenicity
- The identification of drug targets and molecules that exhibit antiparasitic activity
- The development of molecular methods for the diagnosis and typing of *Leishmania* species, as well as population genetics

Pastoral Research Institute of Eastern Europe. Using multilocus microsatellite typing technology (MLMT), a collaborative group of researchers discovered a new group of *Leishmania* strains originating from Turkey and Cyprus and able to cause both cutaneous and visceral leishmaniasis. This population has been demonstrated to be genetically different from the populations present in other European countries. The results of this discovery suggest that the epidemiology of leishmaniasis is more complicated than originally thought, and also indicate that previous typing methods may have been ineffective, further supporting the call for a revision of *Leishmania* taxonomy.

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