A course with a difference

Fighting infectious diseases with technology and knowledge-transfer

Arto Tapio Pulliainen, Jost Enninga, Elena Fernández-Arenas & Gareth Griffiths

Around 10,000 years ago, humans began to domesticate animals. This development—along with the domestication of crops—spread throughout Africa, Asia and Europe, and triggered a steady and unabated increase in the population. But this came at a price: humans living in close proximity to livestock became susceptible to new infectious microorganisms, which crossed the species barrier from animals to cause a range of hitherto unknown diseases. Among these was one of mankind's deadliest companions: *Mycobacterium tuberculosis*. Humans only gained the upper hand in the fight against tuberculosis (TB) in the second half of the twentieth century. The enormous growth in wealth and the agricultural revolution that took place in developed countries alleviated poverty and malnutrition, two of the most important risk factors for the disease. But it was the availability of antibiotics that allowed physicians to embark on the worldwide eradication of TB. It seemed that mankind was finally riding itself of one of its most deadly scourges after smallpox and plague.

However, TB is on the rise again. It is still the most significant cause of death by a single infectious agent: a staggering one-third of the world's population is infected with *M. tuberculosis*. According to the World Health Organization (WHO; Geneva, Switzerland), approximately two million people die from the disease every year, and there are nearly nine million new cases.

Only HIV, which kills around three million people annually, has a higher mortality rate. In fact, *M. tuberculosis* and its close relative *M. avium* are an increasingly important cause of death in immunocompromised AIDS patients.

One important factor for the re-emergence of TB is that the microbe has quickly acquired resistance to various antibiotics;

a recent survey of multi-drug-resistant strains of *M. tuberculosis* (MDR-TB) revealed that the epidemic might be larger than previously thought (Aziz *et al*, 2006). At present, treating TB means following a strict drug regimen over several months. This poses enormous problems for poorer countries where antibiotics are often not available and a lack of public healthcare makes it difficult to ensure compliance. In light of the spread of

the bacterium—and its growing resistance—the WHO declared TB a global emergency in 1993. However, despite the fact that more than a decade has since passed, there are no new antibiotics available, let alone a vaccine.

Several initiatives have addressed the public health threat posed by *M. tuberculosis*, but the main problem remains the lack of basic research. Western societies are unwilling to provide the funds needed, whereas scientists in those countries suffering most from TB do not have the equipment, materials, funds or experience to conduct effective research to develop new drugs, vaccines or other ways to fight the disease. In addition, our colleagues in these countries are often not even aware of the state-of-the-art techniques and instruments that are available in leading laboratories. For that reason, three group leaders from the European Molecular Biology Laboratory (EMBL; Heidelberg, Germany) organized a workshop on functional microscopy with a focus on *M. tuberculosis* in Cape Town, South Africa, in November 2005. The aim was not only to teach advanced techniques to the 21 participants—most of whom came from South America, Asia and Africa—but also to make

them aware of what is possible with sophisticated instruments and, possibly, to forge partnerships to tackle infectious diseases.

Tuberculosis is a disease that largely affects the poor: the homeless in developed countries and the down-trodden masses in the developing world. It affects mainly South America, South-East Asia and, in particular, sub-Saharan Africa, a region of the world that is losing the

struggle with the enormous impact of this and other infectious diseases—notably HIV/AIDS and malaria. The high incidence of TB in these regions is attributable to many causes, among them poverty, a lack of effective antibiotics and vaccines, and non-compliance with treatment. Poor nutrition, overcrowding and the vagaries of everyday life for most people in resource-poor regions create a fertile breeding ground for *M. tuberculosis*.

... TB is on the rise again. It is still the most significant cause of death by a single infectious agent: a staggering one-third of the world's population is infected with *M. tuberculosis*

Furthermore, early detection and diagnosis—which are crucial for efficiently treating and controlling TB—are frequently unavailable owing to the disastrous state of public healthcare in many countries. “The public health programmes vary enormously, depending on wealth, population and stability. The problem facing African governments is not unique: how to deliver healthcare with inadequate budgets,” commented Paul D. van Helden, Director of the Centre of Excellence for Biomedical TB Research at Stellenbosch University in South Africa.

Despite this grim picture, there is not yet sufficient research into the molecular and clinical aspects of TB with the aim of developing new antibiotics, vaccines or other ways to fight the disease. Although the Western world can afford to throw billions of research dollars into the development of drugs against HIV, erectile dysfunction and depression, it has not made comparable efforts to understand the pathogenesis of *M. tuberculosis*, let alone to develop treatments against it. Progress on tackling TB has been hampered by the so-called 90/10 gap—the fact that 90% of global investments into biomedical research and development by the pharmaceutical industry and governments are targeted against diseases that affect 10% of the world's population, in which TB is not included.

The relative lack of concern about *Mycobacterium*—and the misconception that TB is a treatable disease—have made it more difficult to raise public awareness to a level necessary to put the same efforts into both basic and clinical research,

which might have severe implications. “By deferring the problem now, it will simply come back to haunt us and cost future generations orders of magnitude more,” van Helden commented.

To overcome these basic problems, more than 400 participants—including the WHO, the US and UK governments, and the Bill & Melinda Gates Foundation—launched the Global Plan to Stop TB on 27 January 2006 in Davos, Switzerland (www.stoptb.org/globalplan/). This public-private partnership aims to accelerate social and political action to stop the spread of TB, with a focus on the socio-economic aspects. Indeed, once a society reaches a level of economic performance at which it is able to meet the basic healthcare needs of its citizens, the incidence of TB begins to decrease.

However, it is still necessary to support more basic biomedical research with the aim of developing new diagnostic tools, drugs and vaccines. The identification of MDR-TB further highlights the need to understand the pathogenesis of the disease at the molecular level—in particular, how *M. tuberculosis* survives in its human host. The daily death toll from TB and other infectious diseases means that Africa, South-East Asia and South America cannot wait until research in more affluent countries addresses their problems. But, as we have already pointed out, national funding agencies and pharmaceutical companies in the developed world do not see the funding of this research as their priority. At the same time, primary research at this level is still a pipe dream for the developing countries that are most affected. “In many parts of South America, research in the field of TB is focused basically on epidemiology, including isolation of different strains and genotyping. Studies focusing on understanding the basic mechanisms of TB and other infectious diseases are rare and not well funded by local funding agencies,” said an Argentinian participant in the 2005 workshop. “The main problem is that basic healthcare is sometimes not fully covered so why invest in top-level biomedical research if the basic health needs are not properly covered?”

This lack of resources and infrastructure means that scientists in developing countries face enormous challenges in conducting research (Harris, 2004). “In spite of

political and economical efforts put forward in Latin America to develop science itself and technology as a by-product, it has not been enough to be competitive,” said Gonzalo Beluffo, Assistant Professor of Biochemistry at the Universidad de la República (Montevideo, Uruguay), and one of the workshop participants. “Low salaries plus insufficient funds for science and the brain-drain to the Western world hamper development. As it can be envisioned, if our region loses this human capital, darker ages will arise since the dependency on foreign science and technology will be larger and therefore freedom and independency will be lost.”

Several initiatives have addressed the public health threat posed by *M. tuberculosis*, but the main problem remains the lack of basic research

In addition, scientists in developing countries rarely have access to many basic research tools and reagents, the knowledge of—or experience with—advanced scientific techniques or access to sophisticated instruments. Some public and private initiatives now support the transfer of technology to such countries, and collect and donate used scientific instruments (Reynaud, 2005). But as helpful and laudable as such programmes are, they do not fully address the problem, simply because the donated instruments are often outdated and no longer sufficient to perform the level of scientific research now standard in North American and European laboratories. “It is essential to move sophisticated methodology to Africa. The continent will never progress or achieve until it operates at an international level. Furthermore, many mistakes will be made, if work is done in wealthy countries only and an extrapolation or recommendations are made to developing countries based solely on those results,” van Helden said. “Certainly, potential drug targets should be looked at worldwide. However, I believe that unless we ourselves work on these problems, there will be no ‘ownership’ and pride and not much will happen.” Indeed, it is the scientists in South America, Asia and Africa who are probably best-suited to address the enormous health problems associated with diseases such as TB, because they

experience these diseases on a daily basis and have more of an incentive to become involved in their eradication.

Against this background, Gareth Griffiths, Rainer Pepperkok and Philippe Bastiaens from EMBL organized the workshop on functional microscopy of host–pathogen interactions—supported by the European Molecular Biology Organization (Heidelberg, Germany)—with a focus on *Mycobacterium* in Cape Town in November 2005. The choice of the location was deliberate: TB has the greatest impact in impoverished sub-Saharan Africa, and South Africa suffers tremendously under the double attack of HIV and TB. At the same time, it is the most advanced country on the continent—both in economic and scientific terms—and thus holds the greatest potential to tackle TB and other diseases plaguing the developing world.

The workshop provided the opportunity to introduce new research concepts and technologies into a country that has been an epicentre of the problem, and to broaden the horizons of the participants to the realities of the disease. Beyond that, the workshop exposed researchers from Africa and other countries to highly sophisticated research tools and methods, which they would not have had a chance to work with in their own countries. Finally, the workshop aimed to foster discussions between researchers of different backgrounds, in the hope of leading to collaborative attempts to bridge cutting-edge technological knowledge with clinical reality.

... it is the scientists in South America, Asia and Africa who are probably best-suited to address the enormous health problems associated with diseases such as TB...

Twenty-one young researchers were selected from laboratories all over the world. Approximately one-third worked in Africa, one-third in Europe and one-third in other regions of the world. The vast majority were students or post-doctoral researchers involved in infectious disease research—but for many of them, it was still their first experience with advanced light microscopy.

Practically, the goal of the Cape Town workshop was to teach state-of-the-art light microscopy methods by analysing vesicular transport in mycobacteria-infected macrophages. *M. tuberculosis* and *M. avium*—but not non-pathogenic mycobacteria—are able to survive and grow in what is normally a hostile, highly microbicidal environment: the phagosome of a macrophage. More specifically, pathogenic mycobacteria are able to block phagosome maturation. The mycobacteria-containing phagosomes fuse with early endosomes, thereby acquiring nutrients, such as iron from transferrin, but their fusion with the late endosomes and lysosomes that contain the microbicidal machinery of macrophages is blocked (Russell, 2001; Vergne *et al.*, 2004). This blockage must be mediated, at least in part, by modulation of normal function of macrophage proteins by mycobacteria. The identification of these proteins might act as a starting point for a rational drug design against TB.

In principle, modern light microscopy techniques could be applied—together with genome-wide RNA interference screens—to knock down proteins selectively to answer two main questions. First, which macrophage proteins, when removed from the system, induce maturation of phagosomes containing pathogenic mycobacteria? Second, which macrophage proteins prevent the maturation of phagosomes containing non-pathogenic mycobacteria when removed from the system? These proteins could be considered as regulators of normal phagosome maturation and might be possible drug targets to efficiently kill pathogenic mycobacteria once they invade macrophages.

The logistics involved in organizing such an ambitious workshop would also have been demanding in a European laboratory. However, setting it up in Cape Town required enormous efforts from many people, particularly the local host, Trevor Sewell, Director of the Electron Microscope Unit at the University of Cape Town. Much of the equipment, which was transported from Europe and Japan, entered the African continent for the first time. Leica (Wetzlar, Germany), Olympus (Tokyo, Japan) and Zeiss (Oberkochen, Germany) generously provided top-of-the-range microscopes and technical support. In addition, a custom-made fluorescent life-time imaging microscope (FLIM) was transported from EMBL.

...bringing expertise, sophisticated instruments and advanced techniques to the places where they are needed most could become an efficient way to address the problem of infectious diseases

It was a magnificent achievement that this piece of equipment could be successfully set up on a different continent in only a week—this included finding and transporting a table that weighed about one tonne to prevent vibrations that could disturb the sensitive microscope. The effort was justified by the ability of FLIM to visualize phosphorylation of plasma membrane receptors on living macrophages (see supplementary information online). The biological material—cells, bacteria and supporting materials, including a series of slides on which macrophages could be grown on spots of different RNA interference molecules—were also transported from EMBL and cultured in Cape Town.

The fact that the techniques and microscopes used during the workshop had never before been applied to investigate the fate of mycobacteria-infected cells gave the course a unique flavour: the participants and many of the instructors were not only learning new techniques, but also applying them to experiments with relevance to the real world. Of course, even with a team of 21 students and more than a dozen specialists, there is a limit to how much can be achieved in only a few days. Nevertheless, we succeeded in establishing new approaches that could become helpful not only for analysing mycobacteria but also for studying other pathogens that can survive within human cells (see supplementary information online). This is impressive, given that most participants were hitherto unfamiliar with the techniques taught and applied during the course.

More importantly though, the workshop brought together scientists with different backgrounds and from top laboratories and institutions in the developed and developing worlds, which might lead to new and productive collaborations that could benefit both partners. For example, one workshop participant recently moved from Argentina to EMBL to continue studying *M. tuberculosis*–host cell interactions. His long-term goal is to learn new technologies

and gain knowledge that could be applied to research on infectious diseases in South America.

... simply providing advanced technology is of little help if there is no infrastructure in place to use and maintain it efficiently

Such a workshop, with a clear focus on an important public health problem, could therefore provide a stimulus to improve basic research in developing countries, while offering researchers access to knowledge and technologies that are not available in their own countries. Thus, bringing expertise, sophisticated instruments and advanced techniques to the places where they are needed most could become an efficient way to address the problem of infectious diseases. If so, we believe that research and funding organizations should support such workshops, despite their logistical and other challenges.

Of course, we do not think that a single workshop on advanced technologies will help the developing world to eradicate infectious diseases overnight. One main problem clearly remains the lack of research funds, which the Global Plan to Stop TB might help to overcome, in particular with support from major philanthropic organizations such as the Bill & Melinda Gates Foundation. Another problem is that simply providing advanced technology is of little help if there is no infrastructure in place to use and maintain it efficiently. "Sophisticated assays can only be done in some selected places. One of the problems lies in sustainability. Few people realize this problem and even fewer work towards it and of

those, very few indeed have the track record to attract investment," van Helden commented. "Providing set-up funding is not the answer, unless the people and place where it is established has a track record and someone or a number of people who can drive it."

However, on the basis of our experiences, we would encourage more of these workshops in the future, in conjunction with substantial financial investment in local facilities, equipment and training of personnel. The country of choice is important because certain types of infrastructure should be available. South Africa is a very good example, because its leading universities are able to provide such support. Ultimately, this would result in a sustainable platform of sophisticated technologies run by skilled professionals who are not only spreading the knowledge to nearby regions, but also developing tools to meet the challenge of emerging and re-emerging infectious diseases. In addition, friendships forged during such an international workshop might lead to collaborations and, over time, new therapeutic approaches. Overall, this shows one of the most important features of science: it is not about moving mountains, but about bringing dedicated and creative people together to solve problems that ultimately affect all of us—in this case TB.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>)

REFERENCES

- Aziz MA, Wright A, Laszlo A, De Mynck A, Portaels F, Van Deun A, Wells C, Nunn P, Blanc L, Raviglione M (2006) Epidemiology of antituberculosis drug resistance (the Global Project on Anti-Tuberculosis Drug Resistance Surveillance): an updated analysis. *Lancet* **368**: 2142–2154

- Harris E (2004) Building scientific capacities in developing countries. *EMBO Rep* **5**: 7–11
 Reynaud EG (2005) Scientists for a better world. *EMBO Rep* **6**: 103–107
 Russell DG (2001) *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat Rev Mol Cell Biol* **2**: 569–577
 Vergne I, Chua J, Singh SB, Deretic V (2004) Cell biology of *Mycobacterium tuberculosis* phagosome. *Annu Rev Cell Dev Biol* **20**: 367–394

- Arto Tapio Pulliainen (top left) is at the Biozentrum, University of Basel, Switzerland and the Department of Medical Biochemistry and Molecular Biology, University of Turku, Finland. E-mail: arto.pulliainen@unibas.ch
 Jost Enninga (top right) is at the Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris, France. E-mail: jostenn@pasteur.fr
 Elena Fernández-Arenas (bottom left) is at the Departamento de Microbiología II, Universidad Complutense de Madrid, Spain. E-mail: arenas@farm.ucm.es
 Gareth Griffiths (bottom right) is at EMBL, Heidelberg, Germany. E-mail: griffiths@embl.de

doi: 10.1038/sj.embor.7400907