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Tuberculosis 2012: Biology, Pathogenesis and Intervention Strategies; an update from the City of Light

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Abstract

Tuberculosis (TB) remains one of the world's most deadly infectious diseases, with approximately 1.5 million deaths and 9 million new cases of TB in 2010. There is an urgent global need to develop new control tools, with advances necessary in our basic understanding of the pathogen, *Mycobacterium tuberculosis*, and translation of these findings to public health. It was in this context that the "Tuberculosis 2012: Biology, Pathogenesis, Intervention Strategies" meeting was held in the Institut Pasteur, Paris, France from 11-15th Sept 2012. The meeting brought together over 600 delegates from across the globe to hear updates on the latest research findings and how they are underpinning the development of novel vaccines, diagnostics, and drugs.

Introduction

Paris has a long and distinguished history of international conferences dealing with tuberculosis (TB), dating back to 1867 and the first international conference of Internal Medicine when Jean-Antoine Villemin proposed that TB was a contagious disease (Enarson, 2004). The "Tuberculosis 2012: Biology, Pathogenesis, Intervention Strategies" meeting held at the Institut Pasteur, Paris, from 11-15th Sept 2012 certainly lived up to this proud tradition, with an outstanding array of speakers, over 300 poster presentations, and over 600 delegates. It would be impossible in this short report to cover the breadth of science presented at the meeting in the detail it deserves, but we have tried herein to summarise some of the salient advances. We have structured our review along the same lines as the conference oral sessions, and would like to thank all our colleagues for their permission to present their unpublished work in this format.

Opening session

The opening session set the tone for the following days, mixing basic research with current progress in halting the TB epidemic. Hannu Laang (EC, Brussels) provided insight into the European TB funding landscape, while Christian Lienhardt (WHO, Switzerland) gave an overview of progress in achieving the TB reduction targets set out by the Millennium Development Goals and the STOP TB Partnership. There are clearly some 'good news' stories, with the success of DOTs and the declining global TB incidence rates. However, there are still many problems facing us, including that one third of TB cases are not diagnosed or treated; the emergence of XDR and MDR TB; and the continuing synergy between HIV and TB in Africa. Clearly we are still some distance from a world without tuberculosis.

The role of basic science in providing new approaches to TB control was highlighted by two keynote lectures. Patrick Brennan (Colorado State University, Fort Collins, USA) brought us on a wonderful journey through his 50 years of research on TB and leprosy, highlighting how the work of his group and others has revealed the complexity and biosynthesis of the mycobacterial cell wall. Stewart Cole (EPFL, Lausanne, Switzerland) then presented a journey through TB time and space, weaving together the global dissemination of *M. tuberculosis* with its dissemination through the host, the latter process being controlled in part by the EspR nucleoid associated protein (NAP) (Blasco et al., 2012). The role of EspR in controlling expression of a number of virulence factors reveals the potential of such NAPs as novel drug targets.

Mycobacterial Evolution, Genetic Diversity and Conservation

The application of genome sequencing to *M. tuberculosis* has led to enormous advances in our understanding of the evolution and global dissemination of *M. tuberculosis*. This theme was continued at the meeting, with new data presented by Roland Brosch (Institut Pasteur, Paris, France) on the genome sequences of smooth strains of *Mycobacterium canetii* (Brisse et al., 2006, van Soolingen et al., 1997, Gutierrez et al., 2005). Their analyses demonstrate that, in contrast to comparisons between other *M. tuberculosis* complex members, the smooth strains are genetically more diverse, with high numbers of SNPs and evidence for recombination. The geographical isolation of the smooth TB strains to East Africa may indicate that they are less adept at maintenance in human populations, suggesting that comparative analyses between smooth strains and other *M. tuberculosis* lineages may uncover key traits that led to the latter's global success.

While the relatively low genetic diversity of *M. tuberculosis* isolates is well described, this does not translate into phenotypic homogeneity, a fact emphasised by Stefan Niemann (Borstel Research Centre, Germany). Drawing on an international collection of *M. tuberculosis* lineages, they have shown that there are distinct differences in these strains transcriptomes and interaction with macrophages (Homolka et al., 2010). These lineage specific traits may reflect the evolution of *M. tuberculosis* with its human host, a theme developed by Iñaki Comas (Centre for Public Health Research, Valencia, Spain) who proposed a model for co-evolution between *M. tuberculosis* and humans. He put forward the intriguing suggestion that the emergence of 'modern', highly virulent *M. tuberculosis* is a consequence of increases in human population density over the last 70,000 years, with low virulence *M. tuberculosis* having predominated until the last 10,000 years.

Mycobacterial biology and physiology

The mycobacterial cell wall has long been known to have an array of complex lipids, but defining their exact roles has remained elusive. Christophe Guilhot (IPBS, Toulouse, France) gave an overview of the roles of polyketide-derived lipids in TB biology. He showed evidence for synergistic interactions between lipids such as acyltrehaloses and sulfolipids in virulence, which may explain previous work showing that individual gene knock-outs in the respective biosynthetic pathways did not produce clear attenuation phenotypes in mouse models. The role of polyketide-derived lipids was also addressed by Rajesh Gokahle (CSIR-IGIB, Dehli, India), who is exploring the role of such lipids in mycobacterial biofilm formation and reported that *M. smegmatis pks* mutants make defective biofilms; whether the same holds true for *M. tuberculosis pks* remains to be determined. Regulation of FasI and FasII systems was discussed by Gabriela Gago (IBR, Roasario, Argentina), who described the FasR and MabR regulators of the FasI and FasII systems, respectively (Salzman et al., 2010, Arabolaza et al., 2010)

The application of systems biology approaches to *M. tuberculosis*, with precise, quantifiable data on the abundance of transcripts, proteins, and metabolites, is set to provide an exquisite level of detail on the inner workings of *M. tuberculosis*. Olga Schubert (ETH, Zurich, Switzerland), elegantly demonstrated the power of such systems approaches in her presentation of the '*M. tuberculosis* Proteome Atlas'. The proteome analysis was based on the identification of unique, 'proteotypic' peptides for every *M. tuberculosis* protein that were then used to develop selective reaction monitoring (SRM) assays (Picotti and Aebersold, 2012). This SRM approach opens the way for the precise quantification of *M. tuberculosis* proteins in a complex mixture, such as *M.*

tuberculosis grown in a macrophage, and will provide us with valuable information on the relationship between transcript and protein abundance, protein turnover, etc.

In a meeting that was kicked-off by Patrick Brennan, it was apt that the name of Joseph Bigger cropped up during the meeting, Bigger having been the Professor of Bacteriology and Preventive Medicine in Patrick's alma mater, Trinity College Dublin, Ireland. Bigger's seminal work on bacterial persisters (Bigger, 1944) was placed in a modern context by John McKinney (EPFL, Lausanne, Switzerland) who presented his use of microfluidics and time-lapse microscopy to monitor single bacterial cells and their response to antibiotic treatment. Using this system they have uncovered a host of novel findings, including how pulsatile expression of *katG* activates isoniazid and hence killing of *M. smegmatis*, showing how stochastic gene expression across a population is one mechanism that generates variation in bacterial drug susceptibility.

Novel transport systems in Mycobacteria

The disclosure of an outer membrane, the mycomembrane, in the mycobacterial cell envelope revealed another layer of complexity to the transport of products into, and out of, the cell (Hoffmann et al., 2008). The novel Type VII secretion system provides some answers as to how *M. tuberculosis* secretes proteins across this hydrophobic barrier, with *M. tuberculosis* possessing 5 such systems named ESX1-5 (Bitter et al., 2009). However, much work remains to be done to elucidate the exact workings of the ESX mechanism. Daria Bottai (University of Pisa, Italy) has focussed on the ESX-5 system and its roles in secretion of effector proteins from *M. tuberculosis*. Strikingly, the ESX-5 system was shown to be responsible for secretion of a large number of PE and PPE proteins, with loss of the ESX-5 channel protein, EccD5, blocking secretion (Bottai et al., 2012). The ESX-5 secreted PE/PPE proteins were shown to be important immunogens underlining the key role of the ESX-5 system in the host's immune response to *M. tuberculosis* (Sayes et al., 2012). The ESX-5 secretion system was further dissected by Edith Houben (VU Medical Centre, Amsterdam, The Netherlands), who showed that the ESX-5 membrane channel is a multiprotein complex made up of EccBCDE (Houben et al., 2012). The exact configuration of these components is still unclear, but EccE may form the outer pore and hence enable the EccBCDE complex to generate an integrated pore across both the inner membrane and mycomembrane.

The problem of getting nutrients into *M. tuberculosis* was addressed by Alexandre Gouzy (IPBS Toulouse, France) who dealt with nitrogen acquisition. Focussing on the AnsP1 and AnsP2 transporters, which were both originally annotated as putative asparagine transporters, he used a combination of mutagenesis and biochemical characterisation to reveal them to in fact be aspartate (AnsP1) and asparagine (AnsP2) transporters. He then explored the roles of these transporters *in vivo*, and presented compelling evidence that AnsP1 is required during the early stages of infection in mice.

Michael Niederweis (University of Alamaba, Birmingham, USA) concluded this session by discussing the search for porins in *M. tuberculosis* (Niederweis et al., 2010). He presented an intriguing story on the identification of a novel outer membrane channel, MptA, in *M. bovis* BCG and *M. tuberculosis*. As well as its role in solute transport, the results suggested this protein may also function as a secreted toxin, calling to mind secreted autotransporter toxins in *Enterobacteriaceae* (Guyer et al., 2000).

Adaptation to changing environment

A large part of the success of *M. tuberculosis* as a major pathogen relies on its ability to adapt to a variety of environments, to switch its metabolic needs from one source to another, and to resist a number of toxic conditions encountered during infection. A whole session of the meeting was dedicated to mycobacterial adaptation to changing environments, taking examples from processes involved in gene regulation, nutrient assimilation and resistance to host immunity and antibiotic treatment.

The adaptation process in *M. tuberculosis* requires fast and subtly controlled mechanisms to regulate gene expression. Kristine Arnvig (NIMR, London, UK) described the contribution of non-coding RNAs to shaping the *M. tuberculosis* transcriptional landscape (Arnvig and Young, 2012, Arnvig et al., 2011, Arnvig and Young, 2009). Using RNA sequencing, she showed that small non-coding RNAs are differentially expressed by *M. tuberculosis* in various environments, such as in *in vitro* culture, macrophages or mice. Nearly a fifth of the RNA sequencing reads map to intergenic regions in *M. tuberculosis*, a fraction that reaches over 50% in stationary phase conditions as a result of the accumulation of a highly abundant non-coding RNA, MTS2823. A potential role for these regulatory elements in *M. tuberculosis* adaptation to stress and virulence was suggested, as has been reported in many bacterial pathogens (Toledo-Arana et al., 2007); however, the exact targets and signals involved in non-coding RNA-mediated gene regulation in *M. tuberculosis* still remain to be understood. In addition to genetically encoded modulation of gene expression, Babak Javid (Tsinghua University, Beijing, China) proposed that random mistranslation might also be involved in *M. tuberculosis* physiology and resistance to antibiotics. In particular the involvement of this ‘error generating process’ in phenotypic resistance to rifampicin was reported.

Nutritional aspects in *M. tuberculosis* infection were explored from the viewpoint of vitamin capture and utilization. Digby Warner (University of Cape Town, South Africa) studied the role of the co-factor vitamin B_{12} in the physiopathology of *M. tuberculosis*. Screening of a whole-genome insertional mutant library allowed the identification of an ABC-type transport protein that is essential for vitamin B_{12} import *in vitro*. Interestingly, while *M. tuberculosis* is predicted to encode a complete pathway for *de novo* vitamin B_{12} biosynthesis, a previous study had shown that a mutant in the B_{12} transporter was attenuated in mice during the chronic phase of infection. Further research is required in order to establish a causal link between the observed phenotype and a defect in vitamin B_{12} uptake, however these results suggest the potential importance of specific nutrient acquisition systems in *M. tuberculosis* virulence.

In addition to nutrient scavenging, *M. tuberculosis* has to resist hostile environments generated by the host immune system. At the same time, the immune system must balance the need for bacterial control with avoidance of inflammatory-induced immunopathology. Illustrating this point, Christopher Sassetti (Univ. Massachusetts, Worcester, USA) elaborated on the role of nitric oxide (NO) production by macrophages during the course of *M. tuberculosis* infection. While the production of NO upon cell activation by IFN-γ is required to kill intracellular bacteria, he disclosed that NO plays also a critical role in controlling inflammation, and in particular in inhibiting both activation and IL1β maturation, and early neutrophil recruitment to the site of infection (Mishra et al., 2012). This suggests that, unexpectedly, IFN-γ may have a role in the control of inflammation through NO production, in addition to its well-known function in activating the immune system.

The search for novel antigens and correlates of protection

The development of knowledge-based and adapted intervention strategies, including prognostic tools, new drugs and more effective vaccines, to combat TB would benefit considerably from improvements in our understanding of latent infection. To begin the session, Camille Locht (INSERM, Institut Pasteur de Lille, France) summarized his work on the heparin-binding haemagglutinin (HBHA). Disruption of the *M. tuberculosis hbha* gene significantly affects mycobacterial interaction with epithelial cells and impairs extrapulmonary dissemination of *M. tuberculosis* in the mouse (Pethe et al., 2001). HBHA is also a powerful latency antigen, and induces high levels of IFN-γ secretion by the peripheral blood lymphocytes of latently infected subjects (Hougardy et al., 2007). In TB patients, HBHA-specific IFN-γ responses are very low, probably due to the induction of HBHA-specific regulatory T cells during active TB; nevertheless, the response increases after chemotherapy. IFN-γ responses to HBHA may thus contribute to protection against the disease. An approach using a combination of BCG and HBHA as a booster for teenagers and adults or as a post-exposure vaccine was then discussed (Corbiere et al., 2012).

Cecilia Lindestam Arlehamn (La Jolla Institute for Allergy and Immunology, USA) presented results of a genome-wide screen for HLA class II epitopes from *M. tuberculosis*, where 74% of the identified antigens had not been previously described as sources of CD4 T cell epitopes. The antigens identified were derived from almost every protein category, but with an overrepresentation of PE/PPE and Esx proteins. Using PBMC from human donors latently infected with *M. tuberculosis*, she went on to show that the response to *M. tuberculosis* is highly heterogeneous and confined to the CXCR3+CCR6+ memory Th1 cell subset.

Latently infected individuals constitute a hidden disease reservoir, and an improved knowledge of key host and pathogen factors controlling TB latency would greatly benefit TB control efforts. Gilla Kaplan (University of New Jersey, Newark, USA) emphasised the utility of the rabbit as a model of TB latency, using two *M. tuberculosis* strains to illustrate the host-pathogen dynamic. Rabbits infected with the *M. tuberculosis* CDC1551 strain show limited pathology, temporal decreases in TNF production, and control but not eradication of the pathogen. The bacilli can be reactivated following immune suppression, underlining the generation of a state of latency. On the contrary, rabbits infected with the *M. tuberculosis* HN878 strain develop an abundant fibrosis with extensive tissue damage driven by sustained TNF production. This model therefore offers a route to defining key determinants of protective immunity and latency (Subbian et al., 2012).

Biomarkers could facilitate rapid diagnosis and treatment of TB patients and allow preventive measures for latently infected individuals. Stefan Kaufmann (MPIIB, Berlin, Germany) reported on whole-blood gene expression profiling of pulmonary TB against other chronic inflammatory diseases such as sarcoidosis (Maertzdorf et al., 2012). This elegantly displayed the power of such global profiling approaches to define novel diagnostic biosignatures of TB infection. He then went on to discuss the miRNA components of the transcript signature that distinguishes TB from latent infection. In particular, miRNA-223 (a negative modulator of neutrophils) was differentially expressed during active

TB, with miRNA-223 knockout mice highly susceptible to *M. tuberculosis* infection (A. Dorhoi et al., submitted).

Novel vaccine strategies

BCG confers variable protection against pulmonary TB in adults, demanding that new vaccines with better protection are developed. Carlos Martin (University of Zaragoza, Spain) gave an update on the development of a live attenuated vaccine based on the inactivation of two *M. tuberculosis* genes: *phoP*, a transcriptional regulator of key virulence factors in *M. tuberculosis,* and *fadD26* involved in fatty acid metabolism (Nambiar et al., 2012). This vaccine called MTBVAC, has been demonstrated to be safe and protective in animal models and is now progressing to Phase I clinical studies. Leander Grode (Vakzine Projekt Management, Hannover, Germany) reported the results from the Phase I clinical trials of VPM1002, a urease-deficient recombinant BCG secreting listeriolysin (Grode et al., 2005). No serious adverse reactions have been observed, and characterisation of the immune response showed induction of greater polyfunctional T-cells and IFN-γ production in those vaccinated with VPM1002 compared to those receiving BCG. While the latter approach used BCG expressing listeriolysin, Yuelan Yin (Yangzhou University, Yangzhou, China) took the converse approach with *Listeria monocytogenes* expressing an Ag85B-ESAT-6 fusion protein. Subcutaneous, intraperitoneal and intravenous vaccination routes with this recombinant *Listeria* were shown to generate specific Th1-type cellular immunity.

Peter Andersen (Statens Serum Institut, Copenhagen, Denmark) explored a different strategy to enhance BCG efficacy. The H56 subunit vaccine is composed of early, constitutive and late MTB antigens, namely Ag85b, ESAT-6 and Rv2660c (Aagaard et al., 2011). Boosting BCG with H56 resulted in efficient containment of *M. tuberculosis* infection in macaques and prevented reactivation of latent infection (Lin et al., 2012). Helen McShane (University of Oxford, UK) finished this session with another strategy to boost BCG. She reported on the Phase I/IIa studies with the candidate TB vaccine Modified Vaccinia virus Ankara expressing Antigen 85A (MVA85A) from *M. tuberculosis* in healthy volunteers previously vaccinated with BCG (Scriba et al., 2012, Pathan et al., 2012). Two phase IIb trials in BCG-vaccinated infants and in HIV+ adults are currently on-going.

Biochemistry and chemical biology I and II

Adaptation of *M. tuberculosis* to its environment, and most particularly to the phagosomal compartment of the macrophage, is an essential component of its pathogenesis, transmission, and maintenance in the host (Schnappinger et al., 2003). Post-translational modifications, such as reversible acetylation or phosphorylation, are emerging as an important mechanism used by mycobacteria to adjust to environmental changes. John Blanchard (Albert Einstein College of Medicine, New-York, USA) reported that reversible acetylation by a cAMP-dependant protein lysine acetyltransferase reduces the activity of enzymes such as the central metabolic enzyme acetyl-CoA synthase (Xu et al., 2011); MbtA, an adenylating enzyme that catalyses the first step in the biosynthesis of the mycobactins; or FadD26 involved in phthiocerol dimycocerosate synthesis. Marco Bellinzoni (Institut Pasteur, Paris, France) detailed how the PknG substrate GarA, an FHA-domain protein, acts as a switch in central metabolism. Using combined structural and biochemical assays, it was shown how unphosphorylated/phosphorylated GarA controls the activity of a pivotal set of core enzyme, α ketoglutarate dehydrogenase, glutamate dehydrogenase and glutamate synthase, to direct metabolic flux towards the accumulation of glutamate (Wagner et al., 2011, O'Hare et al., 2008).

Laurent Kremer (CNRS, Université de Montpellier, France) highlighted the role of Ser/Thr phosphorylation in the regulation of cell envelope biosynthesis by modulating the activity of enzymes involved in mycolic acid, peptidoglycan or glucan synthesis; of the MmpL7 phthiocerol dimycocerosate transporter; and of the transcriptional repressor EmbR that regulates the expression of arabinosyltransferases involved in arabinogalactan and lipoarabinomannan synthesis (Molle and Kremer, 2010). However, a number of questions still remain, such as the precise environmental signals that are sensed by acetylation/deacetylation and phosphorylation/dephosphorylation systems, if these systems are coordinated, and what physiological advantages are gained by these post-translational modifications.

Functional assignment of all enzymes encoded by the genome of *M. tuberculosis*, and thus an understanding of its complete metabolic capacity, is far from complete. However Luiz Pedro de Carvalho (NIMR, London, UK) is endeavouring to fill this gap in our knowledge by using activity-based metabolomic profiling to permit unbiased discovery of enzymatic activities encoded by genes of unknown function. This approach applies liquid-chromatography mass spectrometry (LC-MS) to analyse the impact of a recombinant enzyme using a cellular extract as a physiological library of potential substrates and products (de Carvalho et al., 2010). In this way, the orphan enzyme encoded Rv1692 was assigned as a D-glycerol 3-phosphate phosphatase. Emilie Layre (Brigham and Women's Hospital, Boston, USA) used a recently developed lipidomic methodology consisting of LC-MS analysis and bioinformatic tools for the broad and unbiased comparative analysis of *M. tuberculosis* total lipid extracts (Layre et al., 2011) to gain insight into the function of the type-III polyketide synthase Pks10. When comparing wild-type, deleted or over-expressing *M. tuberculosis* strains, she identified a new family of branched chain lipids produced by the *pks10* over-expressing strain only, the precise structure of which is currently under investigation.

The rise in antibiotic-resistant *M. tuberculosis* emphasizes the need for new anti-TB agents with novel mechanisms of action. The meeting was very exciting in this aspect with an impressive amount of new anti-TB compounds and targets identified. It is worth noting that most of these *in vitro* or *in vivo* active compounds were obtained by screening chemical libraries on whole *M. tuberculosis* cells, in either replicating or non-replicating stages or inside phagocytes. As a perfect example of the strategies used, Carl Nathan (Weill Cornell Medical College, New York, USA) gave an overview of the first results obtained in a systematic whole-cell screen for compounds that kill non-replicating *M. tuberculosis*, replicating *M. tuberculosis*, or both (Gold et al., 2012). Over 2.5 million compounds provided by academic and industrial partners are due to be tested through funding from the Bill and Melinda Gates Foundation TB Drug Accelerator programme. Confirmed results from 113,000 compounds revealed that 0.5% of the compounds tested killed replicating *M. tuberculosis* selectively, 0.1% killed non-replicating *M. tuberculosis* selectively, and 0.03% killed both. Among compounds that showed bactericidal activity against *M. tuberculosis*, no activity against other bacteria and fungi, and limited toxicity to human hepatoma cells, were certain synthetic analogs of phytopyranocoumarins (calanolides) synthesized by P. Zheng and G. Liu of Tsinghua University. These latter compounds were found to be equipotent against replicating and non-replicating bacilli, had a bactericidal mode of action, and killed bacilli residing in human macrophages. Their target awaits identification.

Mycobacterial resistance and the need for new drug targets- Search for novel drug candidates to cure TB

Erik Böttger (University of Zurich, Switzerland) opened this session with a discussion of drug resistance in *M. tuberculosis*, from the viewpoints of clinical implications and fitness costs. The significant heterogeneity of *M. tuberculosis* drug resistance was one of the key messages of this presentation, i.e. low, medium and high level antibiotic resistance. The case was made for quantitative measures of drug resistance to complement the standard critical concentration methods that are currently used to define resistance (Bottger, 2011). Indeed, the mechanism of drug resistance for TB drugs remain to be clarified, and it is surprising to think that the precise mechanism of action of pyrazinamide (PZA), introduced 6o years ago and one of the cornerstones of modern TB drug therapy, remains to be defined. Ying Zhang (Johns Hopkins University, Baltimore, USA & Fudan University, Shanghai, China) reported the recent identification of RpsA as a novel target for PZA. RpsA acts in concert with tmRNA in trans-translation, a mechanism for releasing partially synthesized proteins from stalled ribosomes, a process of central importance to bacterial persisters; pyrazinoic acid binds to RpfA, blocking its ability to function in trans-translation (Shi et al., 2011).

Priscille Brodin (INSERM, Institut Pasteur de Lille, France) highlighted the potency of high-content cell-based screening and unbiased large scale phenotypic assays for the identification of novel host and mycobacterial genes involved in *M. tuberculosis* intracellular colonization (Figure 1), as well as chemicals able to prevent bacterial intracellular growth (Christophe et al., 2010). During one of these screens in infected macrophages, ImidazoPyridine Amides (IPAs) were identified as potent drugs active against *M. tuberculosis*. As shown by Ulf Nehrbass (Institut Pasteur Korea, Seongnam, Republic of Korea), optimized IPAs inhibit the growth of MDR and XDR clinical isolates in the low nanomolar range. IPA Q203, despite being static *in vitro*, is cidal in the mouse model of *M. tuberculosis* infection, where it accumulates in the lung tissues and inhibits granuloma formation.

The pathway leading to the biosynthesis of mycolic acids is for the most part unique to mycobacteria and the utility of this as a drug target is clearly demonstrated by the therapeutic efficacy of several key anti-TB agents, such as isoniazid and ethionamide, as well as various other compounds, including thiolactomycin, isoxyl and thiacetazone. The primary mode of action of the latter two compounds was unknown, but Mary Jackson (Colorado State University, Fort Collins, USA) described the discovery of a common mechanism of action, i.e. inhibition of the dehydratase step of the Fatty Acid Synthase type II (FAS-II) elongation cycle, catalysed by the (3R)-hydroxyacyl-ACP dehydratases encoded by *hadABC* (Grzegorzewicz et al., 2012). Moreover, she reported the identification, thanks to a whole-cell *M. tuberculosis* screens, of an adamantyl urea compound that shows potent bactericidal activity *in vitro* and that had a unique mode of action, namely the abolition of the MmpL3 translocation of mycolic acids from the cytoplasm, where they are synthesized, to the periplasmic side of the plasma membrane (Grzegorzewicz et al., 2012). Three additional independent studies have recently validated MmpL3 as a new and promising target, reporting compounds that target MmpL3 and inhibit *M. tuberculosis* growth (Tahlan et al., 2012, Stanley et al., 2012, La Rosa et al., 2012).

Benzothiazinones are highly active compounds that were previously shown to target the enzyme decaprenylphosphoryl-β-D-ribose 2'-epimerase and kill *M. tuberculosis* by blocking the synthesis of the arabinan domain of arabinogalactan and lipoarabinomannan (Makarov et al., 2009). Based on the study of the metabolism, pharmacokinetics and mechanisms of resistance to two advanced compounds, Vadim Makarov (Bakh Institute of Biochemistry, Moscow, Russia) reported the design and synthesis by classical approaches of medicinal chemistry of a second generation of benzothiazinone derivatives with improved activity and bioavailability in mice.

Although phenotypic-based approaches provided the most spectacular results, three speakers demonstrated promising target-based strategies to identify new anti-TB drugs. Helena Boshoff (NIH, Bethesda, USA) targeted the non-redundant fumarase enzyme acting in the reverse branch of the TCA cycle as they recently found that fermentation might play an important role in the maintenance of *M. tuberculosis* under the low oxygen tensions encountered in granulomas (Watanabe et al., 2011). *In silico* screening provided sulphonamides as lead compounds, inhibiting the recombinant enzyme and showing activity against anaerobic bacilli, that although moderate, validates the approach. Michael Zimmermann (ETH, Zurich, Switzerland) demonstrated that fermentative activity seems to be a common mycobacterial feature under anaerobia, but that the usage of metabolic pathways differs significantly between fast- and slow-growing mycobacteria.

Owing to the challenge in identifying and validating new targets, David Sherman (Seattle Biomedical Research Institute, USA) reconsidered targets/pathways validated in other organisms but under-exploited in TB chemotherapy, and gave the example of the folate pathway (Kumar et al., 2012). He described several compounds that inhibit *M. tuberculosis* dihydrofolate reductase and that synergize to kill the bacilli by depleting them of methionine derivatives. As an alternative approach to the use of antibiotics, Lydia Tabernero (University of Manchester, UK) proposed targeting a virulence factor from *M. tuberculosis*, MptpB, that is a secreted phosphatase critical in pathogenesis and survival of the bacilli in infected macrophages and animal models (Beresford et al., 2009). The use of a combination of structure-based and computational fragment screening permitted a new family of inhibitors to be designed with remarkable selectivity over human phosphatases and that impair bacillary growth inside macrophages.

Host-pathogen interactions I

As stated previously, one of the main virulence features of *M. tuberculosis* is its capacity to manipulate phagosomal maturation and inhibit the biogenesis of bactericidal phago-lysosomes. While *M. tuberculosis* was thought to exclusively reside within phagosomes inside host-cells, the team of Peter Peters (Netherland Cancer Institute, Amsterdam, The Netherlands) has demonstrated, using electron microscopy, that *M. tuberculosis* and, more generally pathogenic mycobacteria, can be visualized as free bacilli inside the host cell's cytosol (van der Wel et al., 2007). Translocation from the phagosome to the cytosol of the host cell was shown to be dependent on the ESX-1 type VII secretion system, as its introduction to the attenuated vaccine strain *Mycobacterium bovis* BCG, that naturally lacks ESX-1, is needed to trigger translocation. Among ESX-1 secreted proteins, the 6 kDa Early Secreted Antigenic Target (ESAT-6) seems to play a main role in this process, as a mutation in the C-terminus of this protein impairs translocation (Houben et al., 2012). The overall molecular organization of the ESX-1 system in *in vitro* cultivated *M. tuberculosis* was determined by immuno-precipitation techniques followed by 3-D modelling of the ESX-1 proteins EspB and EccA. In this context, Roxane Simeone (Institut Pasteur, Paris, France) presented recently published data where, using single-cell FRET imaging and automated fluorescence microscopy, mycobacterial translocation was quantified and the role of ESX-1 secretion system confirmed (Simeone et al., 2012).

David Russell (Cornell University, Ithaca, USA) reported complementary approaches to probe the micro-environment in which mycobacteria reside during infection, and to exploit this information in order to identify new antituberculous drugs. Using recombinant *M. tuberculosis* strains, in which GFP is expressed under the control of selected promoters responding to well defined stimuli, such as the *aprABC* promoter (Abramovitch et al., 2011), direct temporal and spatial observation of *M. tuberculosis* acidic resistance or cholesterol utilization was possible in mouse tissues. A beautiful example of such probing of the host-pathogen interplay was provided using *M. tuberculosis* strains where GFP was expressed under the control of the hypoxia- and NO-induced *hspX* promoter. Strikingly, fluorescence of the strain was greatly decreased in KO mice impaired in NO production. Using a high-throughput technology based on automated confocal microscopy, a library of chemical compounds was screened in order to identify compounds able to selectively kill bacteria inside macrophages. This method led to the identification of a hundred of promising hits, some of which were exclusively active against intracellular *M. tuberculosis.*

As a complement to the search for new drugs, Anna Coussens (NIMR, London, UK and University of Cape Town, South Africa) presented a fascinating study where molecules were searched for that could potentiate the well-established protective effect of Vitamin D3 against TB. Sodium 4-phenylbutyrate (PBA) was shown to synergise with Vitamin D3 and kill intracellular *M. tuberculosis* through increasing the expression of antimicrobial peptides cathelicidin and lactoferrin. Moreover, PBA was found to significantly restrict *M. tuberculosis* growth in axenic conditions, thereby exhibiting a double level of antibacterial activity. A combination of vitamin D and PBA was proposed as an adjunct to current treatment as means of shortening antituberculosis therapy (Coussens et al., 2012).

Host-pathogen interactions II

Host-pathogen interaction is a finely balanced equilibrium between host defence mechanisms and bacterial survival strategies. Olivier Neyrolles (IPBS, Toulouse, France) illustrated the role of zinc intoxication in macrophages on the outcome of *M. tuberculosis* infection. Following *M. tuberculosis* phagocytosis, an intracellular pool of free zinc was shown to localise to the *M. tuberculosis* phagosome inside primary human macrophages. As high concentrations of zinc are toxic to *M. tuberculosis*, the identification of a bacterial detoxification system was sought. The P-ATPase CtpC was proposed to contribute to zinc detoxification through zinc efflux, as a mutant in the *ctpC* gene was more sensitive to zinc *in vitro*, and was impaired in multiplication inside macrophages. Zinc accumulation in vacuoles is not restricted to *M. tuberculosis* as it is also observed in *E. coli* and latex bead phagolysosomes. Together with similar mechanisms described for copper (Ward et al., 2010, Wolschendorf et al., 2011, Festa et al., 2011) this work reveals a new and unsuspected role for transition metals in innate defence against microbes (Botella et al., 2011) (Figure 2).

Dictyostelium is a practical model to study host-pathogen interactions, as this unicellular organism is easily tractable at the genetic level. Taking *Mycobacterium marinum* as a model pathogen, Monica Hagedorn (Bernhard Nocht Institute, Hamburg, Germany) could show the existence of a mechanism for nonlytic ejection of cytosolic mycobacteria that was responsible for cell-to-cell bacterial transmission. This mechanism is mediated by the formation of a specific and short-lived actin structure called an 'ejectosome' at the plasma membrane of infected cells. In a *Dictyostelium* strain lacking RacH, a small GTPase involved in actin cytoskeleton regulation, no ejectosomes were detected and mycobacterial cell-to-cell spread was drastically reduced. Ejectosome formation appears to be dependent on the expression of the ESX-1 secretion system as no ejectosomes were observed in an *M. marinum* mutant lacking ESX-1 (Hagedorn et al., 2009).

While the major role of type II interferon (IFN-γ) in immune control of *M. tuberculosis* is well established, the role of type I IFN remained incompletely understood until recently. To address this, Ludovic Desvignes (NYU School of Medicine, New York, USA) explored the specific role of type I IFN in the outcome of *M. tuberculosis* infection *in vivo*. Using mice carrying null mutations in the type I IFNR, the type II IFNR, or both, the authors could demonstrate that in a type II

IFNR-null background, the absence of type I IFN increases lung inflammation and causes earlier death of the animals following *M. tuberculosis* infection. While both IFNs are required for optimal recruitment of NK and myeloid cells to the site of infection, the lack of type I IFN specifically increases the number of infected macrophages in the lungs. This study confirmed the major role of type II IFN in restricting mycobacterial growth and revealed a significant function for type I IFN in limiting infectious site expansion (Desvignes et al., 2012).

Using the zebra fish and *M. marinum* as a host-pathogen model system, Lalita Ramakrishnan (University of Washington, Seattle, USA) presented data showing the positive role of the granuloma in mycobacterial colonisation of the host. Through secretion of ESAT-6, *M. marinum* can induce the expression of matrix metalloproteinase–9 (MMP-9) by epithelial cells, which in turn enhances macrophage recruitment (Volkman et al., 2010). The recently reported role of the *lta4h* locus, coding for a leukotriene hydrolase, in balancing host defence *vs.* inflammation-mediated tissue injury was discussed*,* with genetic-associated treatment of TB patients proposed (Tobin et al., 2010).

Animal models and beyond

There is an obvious need for appropriate and robust animals models in which to investigate the fundamental interactions between *M. tuberculosis* and its host, as well as providing the means for testing the efficacy of new drugs and vaccines. Jacques Grosset (John Hopkins University, USA & KwaZulu-Natal Research Institute, ZA) explored this theme by investigating the mode of action of pyrazinamide, a key sterilizing drug in the treatment of TB, and its proposed immune-modulatory mechanism of action (Mendez et al., 2009). Experiments were conducted in immune-competent BALB/c and in athymic nude mice using both *M. tuberculosis* and *M. bovis* (the latter strain being naturally resistant to pyrazinamide). In these models the results were not conclusively able to rule in or rule out an immune-modulatory activity for pyrazinamide, suggesting that further work is needed to clarify this question.

M. tuberculosis infection results in a spectrum of granuloma types, but the mechanisms underlying granuloma formation and maintenance are unclear. Joanne Flynn (University of Pittsburgh, USA) took advantage of PET/CT imaging to study granuloma dynamics in macaques infected with *M. tuberculosis*. In particular, she showed that each granuloma starts with a single bacterium and has a unique trajectory in terms of size and inflammatory changes over time. Interestingly, the percentage of T cells within the granuloma that produce cytokines in response to mycobacterial antigen is very low, with only alternatively activated macrophages found throughout the granuloma, whereas classically activated macrophages are found only around the caesum.

Ludovic Tailleux (Institut Pasteur, Paris, France) has been deciphering how *M. tuberculosis* colonizes extrapulmonary sites. He found that upon *M. tuberculosis* infection, human macrophages express numerous factors including VEGF that controls the formation of new blood vessels. Inhibiting angiogenesis strongly decreases the spread of bacteria in murine models of the disease. This unexpected role of macrophages in the dissemination of the bacillus may explain many extrapulmonary forms of TB.

Stéphanie Boisson-Dupuis (The Rockfeller University, New York, USA) defined how genetic predisposition could explain the inter-individual variability to TB susceptibility. As an example, using a candidate gene approach in a cohort of paediatric patients with acute, disseminated TB, she found that 2 patients out of 50 had autosomal recessive complete IL-12RB1 deficiency (Boisson-Dupuis et al., 2011). This finding, and others, goes to show that the outcome of TB infection is a complex interaction that depends both of the genotype of the host as well as that of the infecting *M. tuberculosis* strains.

Concluding Remarks

The need for a deeper understanding of the immune response to infection is essential if we are to understand latent and active disease, and to provide markers for improved diagnosis, treatment monitoring, etc. Towards this goal, Anne O'Garra (NIMR, Mill Hill, London) has used whole blood transcriptomics to identify a transcript signature that differentiates active TB from both latent TB and other chronic inflammatory conditions. The transcript profile was dominated by neutrophil-driven IFN-γ and type I IFN-$\alpha\beta$ signalling, revealing a key role for type I IFN signalling in TB pathogenesis (Berry et al., 2010).

Clif Barry (NIH, Bethesda, USA) then posed the question of how well 'lab-based' predictions of *M. tuberculosis* and its interaction with the host actually translate into what is seen in the clinic. One such prediction was based on the premise that since *M. tuberculosis* persists *in vivo* in a hypoxic environment, drugs acting under such conditions, such as metronizidaole, would prove effective in treating latent TB, and this held true in animal models (Lin et al., 2012). However, clinical trials showed that metronizidole had limited efficacy for preventing reactivation TB. The converse was illustrated by linezolid, an oxazolidinone, which had poor efficacy in mouse models of TB. However, when given to a cohort of XDR-TB patients in addition to their standard drug regimen, linezolid treatment converted a significant portion of the patients to culture negativity, albeit with side effects linked to drug toxicity (Lee et al., 2012). This presentation, entitled 'an inconvenient truth from the clinic' begged the question: just how good are our current models of TB? Perhaps the answer to this question will be found at the next TB meeting in Paris; we look forward to it.

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Figure Legends

Figure 1. A general approach for the discovery of novel anti-TB drugs based on High Throughput/Content Screening on *M. tuberculosis*-infected macrophages.

High throughput screening of chemicals that interfere with the replication of *M. tuberculosis* within macrophages is performed thanks to a phenotypic cell-based assay that uses automated confocal fluorescence microscopy. RAW macrophage cells are infected with *M. tuberculosis* H37Rv expressing GFP (pictures are shown at day 5 after infection). Chemical compounds are first screened at a single concentration; isoniazid (INH) is used as a control. A customized image analysis automatically quantifies several parameters such as the number of host macrophages, the percentage of infected cells, or the average surface area of bacterial aggregates that are then processed in a Primary Component Analysis (PCA). Hits are confirmed by means of serial dilutions, and MICs are determined. Leads are optimized in terms of efficacy, eADME and *in vivo* PK and the target is looked for before efficacy in mouse model of infection is being assessed by CFU reduction in lungs. The example of an IPA derivative developed by Institut Pasteur Korea and INSERM is given, covered by patents No et al. 18/03/2011, WO2011/113606 A1, PCT/EP2011/001345.

Figure courtesy of Priscille Brodin (INSERM, Institut Pasteur de Lille, France).

Figure 2. Metallobiology of the mycobacterial phagosome.

Within macrophages, *M. tuberculosis* is present in the phagosome, which is depleted of iron and possibly manganese by NRAMP1 and accumulates copper and zinc through the action of the copper transporter ATP7A and other as yet unidentified transporters that may belong to the ZnT/SLC30A family of zinc transporters. In response to iron limitation, *M. tuberculosis* produces iron uptake molecules, such as mycobactins (mbt) and IrtAB. In response to poisoning with copper and zinc, *M. tuberculosis* produces the copper efflux molecules CtpV and MctB, and the putative zinc efflux pump CtpC. Due to a likely high level of redundancy among the mycobacterial Ctp transporters, it is possible that other members of this family of P-ATPases are involved in copper and zinc efflux. See (Botella et al., 2012) for a review.

(Figure courtesy of Olivier Neyrolles, IPBS, Toulouse, France)

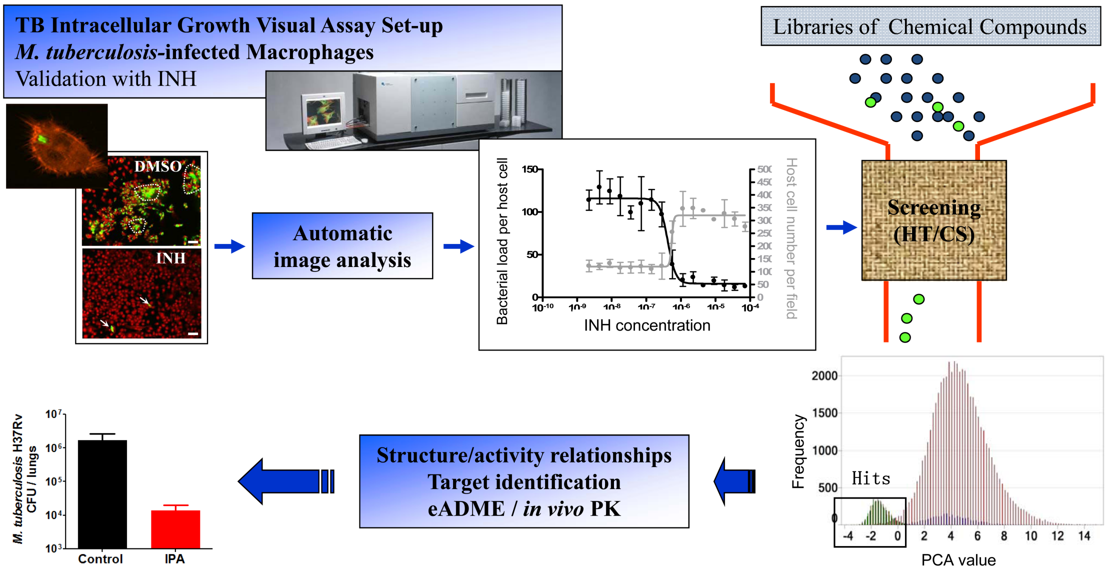
TB Intracellular Growth Visual Assay Set-up
M. tuberculosis-infected Macrophages
Validation with INH
DMSO
INH
Automatic image analysis
Bacterial load per host cell
150
100
50
0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
INH concentration
Host cell number per field
500
450
400
350
300
250
200
150
100
50
0
Libraries of Chemical Compounds
Screening (HT/CS)
Frequency
2000
1500
1000
500
0
Hits
-4
-2
0
2
4
6
8
10
12
14
PCA value
Structure/activity relationships
Target identification
eADME / *in vivo* PK
M. tuberculosis H37Rv CFU / lungs
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
Control
IPA

Figure 1

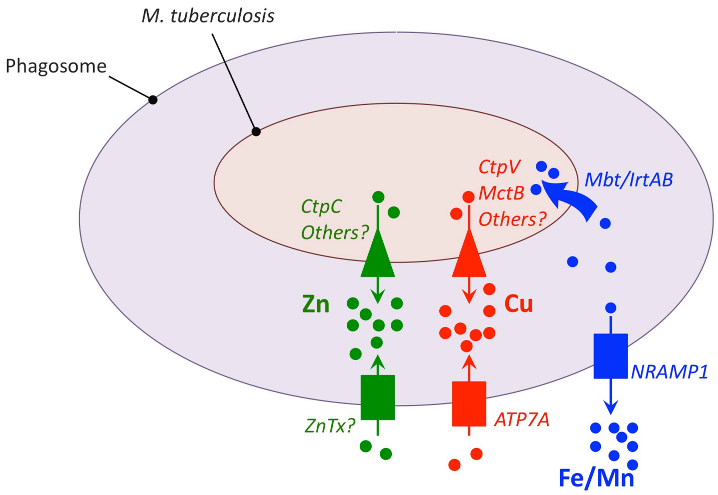
M. tuberculosis
Phagosome
CtpC
Others?
CtpV
MctB
Others?
Mbt/IrtAB
Zn
Cu
NRAMP1
ZnTx?
ATP7A
Fe/Mn

Figure 2