Cutting-edge science and the future of tuberculosis control

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Tuberculosis (TB) is presenting new challenges as a global public health problem, especially at a time of increasing threats due to HIV infection, multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis*. In response to these challenges, the Global Plan to Stop TB advocates the development of new diagnostic tests, drugs and vaccines. In this paper we discuss advances in mycobacterial genetics, functional analyses and related bioinformatics technologies in terms of their potentials for developing tools to address TB control needs.

The genome sequencing of the paradigm strain *M. tuberculosis,* H37Rv, was finished in 1998[1] and sequencing of other subspecies of the *M. tuberculosis* complex and of other mycobacterial species have been achieved or are under way (see: http://www.sanger.ac.uk/Projects/Microbes/), in parallel to an initiative to sequence several clinical *M. tuberculosis* isolates (see: http://www.broad.mit.edu/seq/msc/). Specific genes are potential candidates as targets for development of diagnostic tools or new drugs. Analysis of the complete set of RNA and proteins allows the identification of up- and down-regulated genes and protein expression profiles from bacilli grown in different conditions; for example, in active multiplication or in dormant phase, in macrophages or diseased tissues. Since an extensive description of products currently under development is beyond the scope of this paper (see recent reviews),[2–4] we give some examples to illustrate the impact of the above methodologies for developing new diagnostic tools, new drugs and new vaccines for efficient TB control.

Tuberculosis diagnostics

The current strategy for TB control relies on treatment of patients with active disease. Diagnosis is still performed in most countries by microscopic examination of sputum, a 120-year-old method. The technique is rapid and inexpensive but has a low sensitivity (40–60%), decreasing to 20% in cases of HIV coinfection. Culture is much more sensitive but requires 1–3 weeks from specimen collection, depending on whether liquid or solid culture medium is used. The sensitivity of the technically demanding nucleic acid assays is high for smear-positive specimens, but low (48–53%) for those that are smear-negative. New TB diagnostic tests should be cost-effective and robust enough to be used at peripheral levels of health-care systems, should perform equally well in HIV-infected individuals and should allow sensitive, specific and timely detection of TB cases. Investigation of the complete set of proteins from *M. tuberculosis* under different growth conditions is showing promise for identification of antigens that could be diagnostic for active tuberculosis. Use of bioinformatics allows prediction of protein–protein interactions for identifying proteins that function cooperatively. Moreover, mycobacterial antigens released during active multiplication could be candidates for point-of-care tests, provided that sufficiently sensitive tests can be developed for their detection in blood or, preferably, in urine. In addition, phenolic glycolipids from *M. tuberculosis*, which have long been considered as promising candidates for serodiagnosis of TB, have re-attracted scientific interest and recent studies have started to unravel their complex biosynthetic pathways.[5] These methods, based on the humoral immune response, might be more suitable for the diagnosis of tuberculosis in HIV-infected patients with low CD4+ counts.

Diagnostics of latent infection

Tools for reliable identification of latent TB infections (LTBI) and a predictive risk factor of future progression to active disease are also required for rational use of preventive therapy. The standard test for detecting LTBI is tuberculin skin testing (Mendel–Mantoux) using purified protein derivatives of *M. tuberculosis*. However, problems due to prior immunization with the Bacille Calmette–Guérin (BCG) vaccine or environmental mycobacteria and low sensitivity in immunosuppressed individuals, such as those infected with HIV, underline the need for more specific and more sensitive tests. Progress has been made with the detection of a genomic region encoding two secreted antigens (ESAT-6 and CFP-10) of *M. tuberculosis* that are absent from all BCG strains and from most non-tuberculous environmental mycobacteria. Tests detecting the release of IFN-γ or the presence of IFN-γ-producing cells on contact with ESAT-6 and CFP-10 have been developed and commercialized (QuantiFERON-TB Gold, Cellestis Limited, Carnegie, Victoria, Australia; or T-SPOT-TB, Oxford Immunotec, Oxford, England).[2] Because the antigens are present almost exclusively in tubercle bacilli only, these tests are expected to be more specific for TB infection than the Mendel–Mantoux test. In several low-incidence countries, such tests have been included in the panel of procedures for investigating contacts of persons with infectious TB. However, the potential use of these high-cost tests in endemic countries is still debated.

Antituberculosis drug discovery

The present protocol of antituberculosis treatment requires patients to adhere to a four-drug regimen for a period of 2 months, followed by 4 months of rifampicin and isoniazid. Patients often fail to complete this protocol, which can result in the strain acquiring drug resistance. A primary goal is therefore to develop new molecules that could shorten the length of treatment, are compatible

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with antiretroviral therapy and are effective against MDR-TB and LTBI. At present, fluorquinolone wide-spectrum antibiotics, including moxifloxacin and gatifloxacin, are being evaluated in clinical trials for their potential to shorten the duration of TB treatment.

Moreover, deliberate efforts are being made to identify and develop specific, active antituberculous drugs. Some are already in phase II of drug development. The traditional way of determining the activity of potential new drugs against *M. tuberculosis* is to screen different libraries of new compounds for their ability to act on actively replicating bacteria. Such whole-cell screens are followed by mode-of-action studies to guide drug design. A promising diarylquinoline that has antitubercular activity has been recently discovered using this methodology.[3]

However, target-based approaches also contribute to the identification of new classes of molecules. One of the major challenges thereby is to identify targets that are essential for the bacterium and present in all clinical isolates, but which are absent, or significantly divergent, in humans. In this respect, the public availability of genome sequences of more and more organisms (see: http://www.genomesonline.org/) certainly has an important impact on drug discovery research. Screens, which allow the genome-wide identification of genes that are essential for in vitro and/or in vivo growth, have been developed[6] and results indicate that about 15% of the genes from *M. tuberculosis* are essential for its optimal in vitro growth. An extraordinarily large number of mycobacterial enzymes are involved in lipid metabolism. As many of them are essential for in vitro and/or in vivo growth, they also represent highly attractive targets and are receiving considerable attention. Target identification is, however, only the first of the many steps, which include high-throughput screening, hit-to-lead development and lead optimization procedures.

The development of models of latency help researchers understand the physiological mechanisms that enable *M. tuberculosis* to halt replication while remaining viable. The ability of *M. tuberculosis* to act in such a way is the reason why treatment has to continue for an additional 4 months after the initial 2 months of multi-drug therapy. By using nutrient starvation to mimic latency, whole-genome expression profiling has identified hundreds of genes induced during starvation. Deep insights into the mode of action of a nitroimidazole-derived compound with bacteriocidal activity against dormant tubercle bacilli have been gained by the use of microarray-based sequencing strategies.[7]

Vaccines

The BCG vaccine was developed in 1921 but, although efficient at protecting against disseminated forms of TB in children, is unreliable against the pulmonary forms in adults that account for most of the worldwide disease burden. Current strategies to develop new vaccines are directed either at improving the BCG vaccine for preventive purposes or developing a post-exposure vaccine to prevent reactivation of latent infection. It has been estimated that an effective post-exposure vaccine would prevent 30–40% of TB cases.

In recent years, major attempts have been made to improve pre-exposure vaccines consisting of live vaccine alternatives, and to develop novel booster vaccines.[4] All of these approaches have been enriched by the availability of the genome sequence. There have also been numerous efforts to create recombinant BCG (rBCG) vaccines that offer longer-lasting protection. For example, introduction of the RD1 region (encoding ESAT-6 and CFP 10, as described above) results in increased persistence of the rBCG strain. This vaccine induces better protection against disseminated forms of tuberculosis in mouse and guinea-pig models. Another approach, an rBCG that overexpresses antigen 85B, shows increased protective immunity against TB in the guinea-pig model and has been tested in a phase I clinical trial in uninfected human volunteers. Finally, an rBCG strain that expresses listeriolysin O from *Listeria monocytogenes* is awaiting phase I clinical trials. Attenuated *M. tuberculosis* strains that are deleted for the gene encoding the transcriptional regulator PhoP or for the RD1 region and *panCD* genes have been tested in animal models.

Among booster vaccines, MVA85A, a replication-deficient recombinant vaccinia virus that expresses antigen 85A (Rv3804c), has entered phase II clinical trials, while an adenovirus-based vaccine expressing Ag85 recently has entered phase I clinical trials. In addition, several subunit vaccines have been tested, and some of them, such as Mtb72 in adjuvant AS02A or the Ag85B-ESAT-6 fusion protein in adjuvant IC31, have been tested in phase I clinical trials and are awaiting or entering phase II trials. Booster doses may be used either to strengthen the immunity of BCG vaccine to prevent primary disease in novel prime boost strategies or to strengthen immunity in individuals with latent infection for prevention of reactivation.

Different vaccine strategies will probably be required for the various needs that exist within a population where many individuals have been primed with BCG vaccine or environmental mycobacteria, have been infected with HIV, and/or have been infected latently with *M. tuberculosis*.

Having a test as sensitive as culture and as rapid as microscopy, and a treatment that is shorter and able to eliminate dormant bacilli, will have a major impact on TB control. An effective vaccine for both pre- and post-exposure patients, including those who are HIV-positive, will radically change TB's profile at the population level. ■

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