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Q1 Developing whole mycobacteria cell vaccines for tuberculosis: Workshop proceedings, Max Planck Institute for Infection Biology, Berlin, Germany, July 9, 2014

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ABSTRACT

On July 9, 2014, Aeras and the Max Planck Institute for Infection Biology convened a workshop entitled "Whole Mycobacteria Cell TB Vaccines" at the Max Planck Institute for Infection Biology on the grounds of the Charité Hospital in Berlin, Germany, close to the laboratory where, in 1882, Robert Koch first identified *Mycobacterium tuberculosis* (Mtb) as the pathogen responsible for tuberculosis (TB). The purpose of the meeting was to discuss progress in the development of TB vaccines based on whole mycobacteria cells. Live whole cell TB vaccines discussed at this meeting were derived from Mtb itself, from Bacille Calmette–Guérin (BCG), the only licensed vaccine against TB, which was genetically modified to reduce pathogenicity and increase immunogenicity, or from commensal non-tuberculous mycobacteria. Inactivated whole cell TB and non-tuberculous mycobacterial vaccines, intended as immunotherapy or as safer immunization alternatives for HIV+ individuals, also were discussed. Workshop participants agreed that TB vaccine development is significantly hampered by imperfect animal models, unknown immune correlates of protection and the absence of a human challenge model. Although a more effective TB vaccine is needed to replace or enhance the limited effectiveness of BCG in all age groups, members of the workshop concurred that an effective vaccine would have the greatest impact on TB control when administered to adolescents and adults, and that use of whole mycobacteria cells as TB vaccine candidates merits greater support, particularly given the limited understanding of the specific Mtb antigens necessary to generate an immune response capable of preventing Mtb infection and/or disease.

1. Introduction

Q4 **Dr. Stefan H.E. Kaufmann, Managing Director, Max Planck Institute for Infection Biology and Professor of Immunology and Microbiology, Charité Clinics, Berlin, Germany**

The development of an effective vaccination to prevent the spread of tuberculosis (TB) represents an important global health priority. It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb). In 2013, approximately 9 million persons developed active TB, and approximately 1.5 million died of the disease [1]. In 2014, the WHO set a goal of reducing incidence of active TB from the current level of greater than 100 cases per 100,000 persons to 10 cases per 100,000 persons by 2035, and reducing mortality by 95% [1]. Models of disease reduction strategies suggest that current TB control strategies will not be sufficient to reach this goal unless a vaccine capable of preventing TB infection and/or disease becomes available [2–4].

Sixteen different TB vaccine candidates are currently in clinical trials, with more in the preclinical pipeline. Most of these vaccine candidates are subunit vaccines, where selected Mtb antigens are expressed in recombinant viral vectors or are administered as protein/adjuvant combinations [5]. Approximately 12 different antigens are expressed in the subunit vaccines currently in clinical trials. A major challenge to TB vaccine development, however, is the lack of an immune correlate of protection against Mtb infection or TB disease [6]. Accordingly, there is little certainty about the actual protective effect that may be provided by at least some of the antigens currently under investigation in subunit vaccine candidates [7].

Given these concerns, whole mycobacteria cell vaccines are receiving a fresh look as an attractive TB vaccine development strategy [8]. The most familiar whole cell vaccine for TB is Bacille Calmette–Guérin (BCG). Working in the Institute Pasteur in Lille, France, Albert Calmette and Camille Guérin passaged a *Mycobacterium bovis* isolate over a period of 16 years, from 1906 to 1921, eventually developing a strain sufficiently attenuated to administer safely to humans [9,10]. Since its first use in 1921, BCG has become the most widely used vaccine in history, with approximately 4 billion doses administered worldwide [5]. The full effectiveness of BCG

Abbreviations: AFB, acid-fast bacteria; BCG, Bacille Calmette–Guérin; CFU, colony forming units; DAT, diacyltrehalose; DDA, dimethyldioctadecylammonium; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot assay; HIV, human immunodeficiency virus; Hly, listeriolysin O; IFN-γ, Interferon gamma; INH, isoniazid; LAM, lipoarabinomannan; LTBI, latent TB infection; MDR, multidrug-resistant; MGIA, mycobacterial growth inhibition assay; MPL, monophosphoryl lipid-A; Mtb, *Mycobacterium tuberculosis*; NHP, non-human primate; NKT, natural killer T cells; PAT, polyacyltrehalose; PDIM, phthiocerol dipimycocerosate; PPD, purified protein derivative; SATVI, South African Tuberculosis Vaccine Initiative; SL, Sulfolipid; TB, tuberculosis; TNF, tumor necrosis factor; VPM, Vakzine Projekt Management; WHO, World Health Organization; Zmp, zinc metalloprotease.

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vaccination, however, has yet to be accurately determined. There is consensus that BCG administered to infants shortly after birth reduces the risk of severe childhood TB, particularly TB meningitis and disseminated TB. A meta-analysis of the global effect of BCG vaccination on childhood tuberculous meningitis and military TB estimated that in 2002, about 30,000 cases of tuberculous meningitis and 11,500 cases of military TB were prevented by the 100.5 million BCG vaccinations given to infants in that year [11]. Accordingly, the WHO recommends neonatal BCG immunization in countries endemic for TB [11,12]. BCG also may provide children a degree of protection against Mtb infection for a limited number of years after vaccination, although observational studies suggest that this effect is variable and likely is impacted by the latitude in which the child lives, with children in tropical latitudes exhibiting less protection from subsequent childhood infection, possibly due to a higher rate of exposure to other non-tuberculous mycobacteria [13,14].

Despite its widespread use, BCG vaccination has important limitations. Recent studies in BCG-immunized pediatric populations estimates total incident childhood cases of all forms of TB in 2011 to be almost 1 million, twice that estimated by WHO, and provides an initial assessment of multidrug-resistant (MDR) TB cases at over 30,000 [15]. In addition, BCG is not recommended for use in HIV-infected infants because of the risk of disseminated BCG disease [16]. Most importantly, the ongoing global epidemic of TB infection, disease and death among adolescents and adults is occurring in populations in which infant BCG vaccination is nearly universal. Accordingly, developing a more effective whole cell vaccine than BCG represents an important goal of global TB control efforts.

The July 9, 2014 whole mycobacteria cell TB vaccine workshop, convened in the shadow of the laboratory of Robert Koch, discoverer of the Mtb bacillus more than 130 years previously, specifically examined the potential role of whole mycobacterial cells as vaccines to prevent infection and/or disease due to Mtb. The workshop was divided into three sessions: (1) the research and development status of seven whole cell TB vaccines; (2) development pathways for whole mycobacteria cell vaccines, focusing on the rationale for and public health implications of developing replacement BCG vaccines for infants and for using whole cell vaccines to prevent Mtb infection and disease in adolescents and adults; and (3) resource needs for developing whole cell TB vaccines, with a focus on the identification of standardized assays for vaccine immunogenicity and efficacy to permit reliable vaccine candidate comparisons. A summary of the whole mycobacteria cell vaccines for TB discussed in this workshop can be found in Table 1.

2. Status of ongoing whole mycobacteria cell vaccine research and development

BCG + ESAT-6 recombinants. Dr. Roland Brosch, Institut Pasteur, Unit for Integrated Mycobacterial Pathogenomics, Paris, France

Dr. Roland Brosch described efforts by his laboratory to improve the BCG vaccine. Theorizing that BCG may be lacking some important genetic features to provide effective protection against Mtb, Dr. Brosch proposed that vaccine immunogenicity and efficacy may be improved by adding back a gene cluster to BCG that was lost during the BCG passaging and attenuation process. All BCG vaccines lack the RD1 locus, the region of difference 1 [17,18]. The RD1 locus encodes at least 11 genes, including the immunodominant T-cell antigens ESAT-6 (6-kD early secretory antigenic target) and CFP-10 (10-kD culture filtrate protein), both representing important mycobacterial antigens secreted by the ESX-1 type VII secretion system, representing potential Mtb virulence factors. Restoration of the entire RD-1 locus to BCG is required for ESAT-6 secretion and partially restores virulence [18]. When the recombinant BCG, BCG::ESX-1, was compared to the parental BCG for immunogenicity and protection against an Mtb challenge [19], it was shown to be more virulent in immune deficient mice, more persistent in immune competent mice, but also more effective in protecting against disseminated TB in both mice and guinea pigs [18,19].

As a possible explanation for the increased virulence in severe combined immune deficient (SCID) mice and the improved protective vaccine efficacy of the recombinant strain, it was found that ESAT-6 may disrupt lipid bilayers [20,21], thereby providing a mechanism for ESX-1 proficient tubercle bacilli to egress from the macrophage phagosome to the cytoplasm [22,23]. The effect of ESAT-6 on the fate of macrophage-phagocytized BCG also was studied with the fluorescent substrate, CCF-4. BCG is unable to progress from the phagosome to the cytoplasm, while BCG::ESX-1 moves to the cytoplasm with Mtb kinetics. Phagosomal rupture is followed by necrotic cell death of infected macrophages, resulting in BCG::ESX-1spread to new host cells. Access to the cytosol by the recombinant BCG::ESX-1 allows additional immunological responses and higher amounts of antigen, in part also explaining the stronger $CD8^+$ T-cell responses observed for Mtb relative to BCG [24].

A series of recombinant, ESX-1 proficient BCG constructs were developed that retain ESX-1 function but reduce the virulence. Mutation of ESAT-6 leads to less virulent recombinant BCG vaccines that express and secrete modified ESAT-6 antigens which proved to provide better protection in mouse models and to be somewhat more protective in a guinea pig model than the parental BCG Pasteur strain (Bottai and Brosch, unpublished results). The use of different BCG strains as a template for ESX-1 complementation, such as BCG Moreau, also is being explored. Finally, attempts also are being made to use the recombinant BCG::ESX-1 Mar, created using the ESX-1 system from *Mycobacterium marinum*, a biosafety class 2 organism, to complement BCG strains. Preliminary results for this approach are promising (Groschel and Brosch, unpublished results). Dr. Brosch concluded his presentation by emphasizing that the ESX-1 locus encodes genes critical for mycobacterial host pathogen interaction, and that inclusion of this locus in recombinant BCG vaccines is expected to enhance the immune response to the vaccine on multiple levels which seem all to be impacted by the ability of the vaccine to gain access to the host cytosol [25].

VPM1002. Dr. Leander Grode, Vakzine Projekt Management GmbH, Hannover, Germany. Dr. Umesh Shaligram, Serum Institute of India Ltd., Pune, India

Dr. Leander Grode and Dr. Umesh Shaligram jointly discussed progress in scaling up production of the vaccine candidate VPM1002, and shared the results of Phase 1 and Phase 2 clinical trials. VPM1002 employs the pore-forming protein listeriolysin O (Hly) from the facultative anaerobic bacterium *Listeria monocytogenes*, coupled with a urease C gene deletion in a BCG Prague genetic background, to allow antigen to escape from the phagosome into the cytoplasm of the infected cell [26]. The BCG bacteria, however, remain in the phagosome [26]. Acidic pH is optimal for Hly activity; deletion of the urease C gene prevents BCG neutralization of the naturally acidic phagosome environment [27]. A safety feature of this vaccine is the four amino acid PEST sequence (P, proline; E, glutamic acid; S, serine; T, threonine) which targets the Hly protein for rapid degradation in the cytosol [28,29]. Following entry into the cytosol, the Hly protein is rapidly inactivated, thereby reducing the possibility of adverse events due to the pore-forming bioactivity of this protein. Antigen access to the cytosol is presumed to allow for a broader activation of immune mechanisms and improved vaccine efficacy [30–33].

VPM1002 initially was created in the laboratory of Dr. Stefan H.E. Kaufmann [34,35] and was further developed by VPM. In 2013, the rights to the vaccine were acquired by SII, which has developed

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