



Task Force on New Approaches to TB Vaccine Development

Meeting Report

July 1-3, 2007

**Fondation Mérieux
Veyrier-du-Lac, France**



Working Group on New TB Vaccines

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THE STOP TB WORKING GROUP ON NEW TB VACCINES

To facilitate the enormous task of developing a new TB vaccine, the Global Partnership to Stop TB has established a Working Group on New TB Vaccines, which is one of the seven working groups within the Partnership. The other Working Groups include New TB Drugs, New TB Diagnostics, DOTS Expansion, MDR-TB, TB/HIV, and Advocacy, Communications and Social Mobilization (ACSM).

The Working Group on New TB Vaccines seeks involvement of a cross-section of parties interested in accelerating the development and availability of a new TB vaccine. Partners include the private sector, academic institutions, philanthropic organizations, research institutions and non-governmental organizations.

The Working Group exerts its role as a broker and facilitator largely through specific initiatives. These time-limited initiatives are aimed at tackling pressing issues which can only be solved by international consensus-building and collaboration, e.g. (a) harmonization of laboratory assays and clinical endpoints, (b) definition of health–economic parameters and (c) social mobilization strategies, to name just a few.

Objectives and activities of the Working Group include:

- Maintain and improve BCG vaccination programmes
- Discovery and translation research ("keeping the pipeline filled")
- Facilitate preclinical development
- Build capacity at vaccine trial sites
- Ensure availability of vaccine production capacity/scale-up
- Perform clinical trials
- Provide an enabling infrastructure and environment

New Approaches to TB Vaccine Development Task Force Mission

The Task Force on New Approaches to TB Vaccine Development will focus on combining traditional approaches to TB vaccine development with “out-of-the-box” thinking in order to ensure a continuous, robust TB vaccine pipeline and the ultimate development of a successful new TB vaccine.

Task Force Objectives

- To identify fundamentally new approaches to prevention of TB
- To develop mechanisms to attract investigators from other disciplines and specialists on diseases other than TB diseases to develop innovative TB vaccine research proposals
- To provide young TB researchers and PhD students at post-doc level with incentives to develop and test innovative ideas
- To identify funding opportunities/pathways for high-risk research on next generation TB vaccination approaches
- To develop a mechanism to ensure rapid transition of innovative TB vaccine discovery approaches into a standardized vaccine development pathway

TASK FORCE PARTICIPANTS

Jerald C. Sadoff, MD (Chair)
Aeras Global TB Vaccine Foundation
Rockville, Maryland USA

Rafi Ahmed, PhD
Emory Vaccine Center
Atlanta, Georgia USA

Peter Andersen, DVM, DMSc
Statens Serum Institut
Copenhagen, Denmark

Arturo Casadevall, MD, PhD
Albert Einstein College of Medicine of Yeshiva
University
Bronx, New York USA

Camilo Colaco, MD
ImmunoBiology Limited
Cambridge, United Kingdom

David C. Kaslow, MD
Merck Research Laboratories
North Wales, Pennsylvania USA

Douglas Kernodle, MD
Vanderbilt University School of Medicine
Nashville, Tennessee USA

Stefan H.E. Kaufmann, PhD
(unable to attend)
Max Planck Institute for Infection Biology
Berlin, Germany

Paul-Henri Lambert, MD
Center for Vaccinology
Genève, Switzerland

David Lewinsohn, MD, PhD
Oregon Health Sciences University
Portland, Oregon, USA

Karen Lignau, PhD
Intercell AG
Vienna, Austria

Margaret Liu, MD
San Francisco, CA

Steve Reece, PhD
Max Plack Institute for Infection Biology
Berlin, Germany

Graham A. Rook, MD
University College London
London, United Kingdom

Robert Seder, MD
Vaccine Research Center, NIAID, NIH
Bethesda, Maryland USA

Christine F. Sizemore, PhD
(unable to attend)
National Institute of Allergy and Infectious
Diseases, Division of Microbiology and
Infectious Diseases
Bethesda, Maryland USA

Alexander von Gabain, PhD
Intercell AG
Vienna, Austria

David B. Weiner, PhD
University of Pennsylvania
School of Medicine
Philadelphia, Pennsylvania USA

Hans Wigzell, MD, PhD
Karolinska Institute
Stockholm, Sweden

Rolf Zinkernagel, MD, PhD
(unable to attend)
University Hospital Zurich
Institute of Experimental Immunology
Zurich, Switzerland

MEETING SUMMARY

The first meeting of the Task Force on New Approaches to TB Vaccine Development, part of the Stop TB Partnership Working Group on New TB Vaccines, was held July 1-3, 2007 at Fondation Mérieux in Veyrier-du-Lac, France. Presentations and discussion covered a number of new and innovative approaches as well as the current portfolios and state-of-the-art in TB vaccine development. Paul-Henri Lambert described the rationale, approach and components in the TB-VAC pipeline and Jerald Sadoff did this for the Aeras Global TB Vaccine Foundation candidate pipeline. The major approaches include modifications to improve BCG, attenuated Mtb, and subunit vaccines, mainly for boosting BCG, using a variety of recombinant TB proteins with adjuvants and several different viral vectors and other novel approaches for immunization with TB proteins. Rafi Ahmed provided new basic information on protective immune responses to priming and boosting immunizations, identifying important variables that affect the quality of protection. Graham Rook and David Lewinsohn focused on the cellular immune responses in TB with emphasis on ways to deal with misdirection of the immune response so that it will be more favourable to the host and the likely high importance of the CD8+ T cell component of the immune response in humans for protection against TB. Karen Lingnau reported on work at Intercell with new toll-like receptor adjuvants including encouraging early clinical results with their IC31 adjuvant. Peter Andersen described mechanisms of action of cationic liposomes in adjuvanting TB subunit vaccines and promising early-stage work on vaccines against latent TB, particularly with the Gates Foundation Grand Challenge 12 group. David Weiner provided promising information on the contribution of cytokine based adjuvant stimulation to the production of cellular immune responses with DNA vaccines. Additional new concepts including modifications in BCG to make it more pro-apoptotic and thereby more immunogenic and the use of heat-shock protein + cellular protein complexes as immunogens were presented by Douglas Kernodle and Camilo Colaco, respectively. Arturo Casadevall made a compelling case for adding development of TB vaccines inducing antibody to the current approaches which are all cell mediated immunity directed.

Hans Wigzell commented that antibodies are a perfect adjuvant for T cell recruiting and they ought to be looked at related to both protection and disease – passively transferred maternal antibodies may affect BCG responses and disease in infants. He expressed concern regarding shortcomings of animal challenge test systems, particularly mice, suggested that unique antigens may be better than common antigens, and wondered if enough is known about effects of immunization to proceed with advancing clinical trials. In discussion of the current, ethical requirement to include BCG at birth in clinical trials, Paul-Henri Lambert indicated that WHO recently issued conservative advice regarding the use of BCG in children infected with HIV. David Kaslow added a number of practical considerations for development of investigational candidates. Indications in the product label could include prevention of childhood TB disease and latent TB (pre- and post-exposure), prevention of reactivation and adult TB disease (pre- and post-exposure); shortening of drug regimens (post-exposure, adjunct to therapy). Efficacy trial endpoints definitions could include evidence of infection, disease, progression of latent TB to disease, and reactivation of latent TB– measurements would need to be agreed for each. He supported the proof-of-concept strategy for getting to large efficacy testing and strongly supported the need to look hard for biomarkers that correlate with protection and might eventually become surrogates for efficacy.

HIGHLIGHTS OF INDIVIDUAL PRESENTATIONS

State-of the-art in current approaches for TB vaccines

- **TB-VAC candidates pipeline – Paul-Henri Lambert**
- **Aeras Global TB Vaccine Foundation candidates pipeline – Jerald C. Sadoff**

The pipelines of vaccine candidates under development by TB-VAC and the Aeras Global TB Vaccine Foundation include multiple new subunit vaccines (proteins plus adjuvants and vectored by live viruses and Shigella capsids), and genetically engineered live TB vaccines (recombinant, modified BCGs, attenuated Mtb). Presentations provided updates on preclinical and clinical studies of multiple candidates being tested individually and eventually intended in most cases to be used in prime-boost regimens. The heterologous prime-boost strategy, starting with BCG, recombinant BCG or attenuated Mtb and generally followed by one or more subunit vaccines is expected to be a good method for induction of cellular immunity in humans. It is anticipated that priming will occur at or near birth with BCG or rBCG and will be followed by boosting initially in infants and later in adolescents with either recombinant proteins in adjuvants or a viral or capsid vectored vaccine.

For live mycobacterial priming vaccines, potential advantages include relative ease to produce, BCGs experience may help with introduction, initial protection and pre-boost priming, and possible adjuvant effect for other vaccines. Some issues are stability of modified strains, need to be safer than BCG in HIV-infected and immunocompromised individuals, need to be better than BCGs, and some possible clinical trial issues related to current routine use of BCGs as recommended by WHO.

For subunit boosting vaccines, potential advantages include: good for boosting after infant BCG exposure, single dose may be enough (live vectored), safety in immunocompromised recipients and possibility of combining with other vaccines. Some issues for subunits include cost, need for new adjuvants, single use only (viral vectored), and risk of pathology enhancement (Koch phenomenon in TB infected individuals).

What's new in immunology potentially relevant to TB vaccine development

Rafi Ahmed

This report on new immunology research information relevant to TB vaccine development described the phases of the evolving CD8 T cell response after antigen stimulation by acute viral infection and after boosting. Initial effector and memory CD8 T cell formation is followed by effector cell death and progressive memory CD8 T cell differentiation in phases from precursors to long-lived memory CD8 T cells. Of particular interest re implications for vaccination were the variables affecting the CD8 T cell responses to boosting. A stronger primary stimulus requires a longer interval prior to boosting for a strong T cell boost response than does a weaker primary stimulus. Heterologous boosting leads to preferential increasing accumulation of effector-memory T cells, T_{EM}, especially in non-lymphoid tissues, up to a saturation effect when further boosting produces very little further expansion of T_{EM} cells. Chronic infection/persistent antigen with sustained T cell receptor (TCR) engagement is not helpful for T cell boosting and

may result in CD8 T cell dysfunction where effector T cell responses no longer progress to functional memory. The strength of the primary response and duration of new, recombinant BCG vaccines persistence may be important in how they perform in prime-boost regimens.

Redirection of cellular response in TB

Graham Rook

There are several pieces of evidence that the IFN- γ response does not adequately explain immunity in TB and that pre- and post-exposure to environmental mycobacteria cause people to develop mixed Th1, Th2, and Treg responses, particularly in developing countries where environmental mycobacteria are most prolific and helminths can increase the Th2 component of the neonatal response to BCG. Mice bred in Mexico and Brazil are exposed to environmental mycobacteria in the breeding rooms. To cause progressive disease in these mice a high dose challenge (10^5 - 10^6 cfu) is required, and the disease does not progress until IL-4 and TGF- β levels rise. Disease is attenuated in IL-4 knockouts. Low dose challenge (~4000 cfu) causes latent infection. By contrast, even *very* low dose challenge (10-100 cfu) will cause progressive disease in SPF animals bred in a more Northern climate. In these animals IL-4 appears unimportant and the disease is not affected by knocking out IL-4 or STAT-6. Interestingly, human TB that is accompanied by high levels of IL-4 is seen mostly in developing countries close to the equator. It was suggested that people in these zones are like the mice in Mexico and Brazil; they have partial immunity and will develop progressive disease only if that immunity is deactivated by IL-4 and TGF-beta. Therefore there might be two rather different types of TB in man (as in mouse): 1) disease caused by low dose challenge in the immunologically naïve, in whom a raised IL-4 is not a pre-requisite for progression; 2) disease in the partially immune requiring high dose challenge to overcome immunity by driving up IL-4 and TGF-beta. Type 2) is seen in developing countries where BCG fails and challenge doses are high, and might pose a distinct set of problems for the designers of vaccines.

Also described were lessons learned from immunotherapy (post-exposure) experiments with a number of agents in Mtb-infected mice. He noted that even in advanced disease in mice, there was a reduction in bacterial burden (CFU) produced by a variety of agents that affect the immune response, including Hsp 65 DNA vaccine, killed *M. vaccae*, anti-IL-4, inhibitor of TGF- β , 16 α -bromoepiandrosterone, and human i.v. immunoglobulin. Possible modes of action for these “immunotherapy” effects include downregulation of IL-4 and TGF- β (blocking either one downregulates the other), increased CD8+ CTLs that kill Mtb-infected macrophages (may be more important in humans than in mice), and increased TNF and IFN- γ . He recommended that vaccine approaches ought to include different vaccines for different environments and emphasis on CTL targets (lipid or peptide), as well as a “negative” vaccine approach that would work like immunotherapy and downregulate a pre-existing Th2 response.

What’s new in toll-like receptor adjuvants

Karen Lingnau

The novel IC31 adjuvant contains a combination of an oligodeoxynucleotide (ODN1a) and a cationic antibacterial peptide (KLK). It acts as a toll-like receptor (TLR) agonist: KLK interacts with cell membranes to enhance antigen uptake by antigen-presenting cells and ODN1a acts

through TLR9/MyD88 signaling pathway of the innate immune system. In preclinical studies the combination of Mtb vaccine fusion protein antigens with the adjuvant has an acceptable toxicology profile and generates both CD4+ helper T cells and CD8+ cytotoxic T cell responses that are much stronger than responses to the individual antigens without the adjuvant. Clinical trials with IC31 adjuvanted TB vaccine candidates and other antigens have shown a good safety profile and promising immunogenicity.

Cationic liposomes

Peter Andersen

DDA + TDB (mycobacterial cord factor) adjuvanted Mtb antigens give similar cell-mediated immune responses but higher Th2/antibody responses than the antigens combined with IC31. DDA liposome adjuvant targets antigen-presenting cells with enhanced antigen presentation. The cationic vehicle interacts with cell membranes and enhances antigen uptake. Responses include IFN- γ and TNF- α effector cells and later IL-2+ memory cells, perhaps related to antigen persistence and/or to more efficient antigen depot formation.

Vaccines against latency

Peter Andersen

The Gates Foundation Grand Challenge 12 has assembled a large group of collaborators to work on post-exposure TB vaccines against reactivation of latent TB infection. The group is focusing on antigens in TB in stage 3: stable, non-replication persistence, and stage 4: reactivation from latency. Upregulation of dosR, latency associated genes in hypoxic conditions, the hot spot of genes in Rv2650-2661, early secreted CFP-10 and Mtb latency antigens Rv7733, 2029, 2627 and 2628 are examples of targets of particular interest. Rv 2659 (starvation hot spot) is 1st to show good T cell immune response in a latency animal model. A fusion protein vaccine candidate, H56, containing 85B-ESAT6-2660 has shown better performance in late reduction of CFU in challenged animals than several other candidates (HyVac4, ESAT6 or 2660 alone).

What's new in cytokine based adjuvants for vaccine induction of cellular immunity

David Weiner

Use of a DNA antigen delivery platform is enhanced with cytokine-base adjuvants (IL-12 and IL-15) so that potency is now 2nd to Adeno-5 for CTL generation. The current, adjuvanted DNA vaccines improve responder frequency measured by CTL and CD4+ T cell proliferation and memory T cells, and, when used together, antibody responses. Combining cytokine adjuvants with better physical delivery by electro stimulation is giving promising preclinical immunogenicity results. Chemokine genes may help trafficking molecules from DNA vaccines to stimulate mucosal immunity against Mtb.

Pro-apoptotic BCG vaccines

Douglas Kernodle

A simple model to explain much of TB pathogenesis and the deficiencies of BCG was proposed, i.e., an antioxidant-proficient *Mycobacterium* does well in certain mammalian hosts who use

oxidants as signaling molecules in the early immune response. *M. tuberculosis* is able to interfere with the signaling events necessary for the induction of strong immune responses.

BCG vaccines evolved from virulent *M. bovis* and retained the capacity for producing large amounts of antioxidants. Cultivation in vitro probably selected for mutants that grow faster and the duplication of chromosomal regions containing genes encoding certain antioxidants probably gave such mutants a selective growth advantage by the reducing cumulative toxicity associated with the oxidation or peroxidation of cell wall lipids, which comprise about 40% of the dry weight of BCG. The increased antioxidants also made BCGs more immune evasive and less effective in man. Variability in antioxidant production may explain much of the variability in the effectiveness of different BCG daughter strains against pulmonary TB in man.

The strategy of preferring BCG daughter strains based on invasiveness and persistence has failed. The current BCG vaccines have problems with (1) inadequate safety in immune suppressed persons, (2) poor memory T-cell responses, and (3) poor protection against lung disease. We need BCG strains that are more immunogenic. In mice, BCG vaccines modified by reducing the activity of immune-evasive antioxidants were found to: (1) be more attenuated than the current BCG vaccine, (2) induce a population of memory T-cells that are recalled rapidly during subsequent challenge, and (3) confer superior protection against granulomatous lung destruction. In effect, these modifications address the major limitations of the current BCG vaccines against TB, modifying BCG in a manner that should make it a safer and more effective vaccine against pulmonary tuberculosis in man.

CD8 T cell targets

David Lewinsohn

Challenges in the human immune response to Mtb include: (1) Control of Mtb replication is associated with the acquisition of adaptive immunity; (2) Sterilizing immunity has not been demonstrated; (3) Human macrophages cannot kill Mtb; (4) Cellular vaccines are needed that duplicate human latent TB infection. Advantages to recognizing intracellular infection with Mtb include: promotion of Th1 T cell response; macrophage activation via TLR and cytokines (IFN- γ and TNF- α); delivery of anti-bacterial peptides (granulysin); deprivation of a favored environment: (apoptosis and autophagy); immune-surveillance

Mtb-specific CD8⁺ T cells present at high frequency in those infected with Mtb (both active and latent infection); are both classically, and non-classically HLA-restricted; preferentially recognize heavily infected cells, and hence may be a surrogate for bacterial burden and/or disease progression; however, important CD8 antigens remain poorly defined.

Important questions

- Does the CD8⁺ response reflect bacterial burden?
- Is there evidence for transient exposure and/or clearance?
- Is a robust response associated with protection?
- What can be inferred from the phenotype of the CD8⁺ response with regard to disease status?

Work is progressing on CD8 antigen discovery, addressing: frequency and intensity of immunodominance in the human CD8+ repertoire. Using peptide pools reflecting known CD4 antigens, high frequency responses can be detected specifically in those with both active and latent TB infection (Best responses observed in descending order are CFP-10, Mtb 9.8 and 8.4 – 85B not as good as a CD8 CTL antigen). Furthermore, a peptide library reflecting approximately 10% of the Mtb genome has been generated, and used to define novel antigens using Mtb-specific T cell clones (EsxJ, PE9, and PEPGRS42), as well as to define the profile of dominant responses directly ex-vivo. Interestingly, the CD8+ response to Mtb appears to be heavily skewed towards antigens presented by HLA-B alleles. Finally, approximately ½ of the CD8+ response to Mtb is focused on non-classically restricted (HLA-Ib) CD8+ T cells. Initial characterization of the role of HspX in presentation to HLA-E restricted T cells was described. Interestingly, many of the non-classically restricted cells are specific for antigens that appear to be protease resistant, suggesting a mechanism by which these antigens may be favored in the protease rich environment of the phagosome.

Development of novel immunobiological intervention based on heat shock proteins Camilo Colaco

Heat shock proteins (hsps) are molecular chaperones required in protein folding, translocation, assembly and degradation. Mild stress triggers rapid induction of hsps; increasing stress results in apoptosis and, under extreme conditions, necrosis. Hsps are always found complexed with cellular proteins as HspCs which can act as non-specific danger signals and also as specific antigen-capture vehicles resulting in antigen presentation and cross-presentation. Mtb and BCG HspC experimental vaccines protect mice against aerosol challenge; protection correlates with cell-mediated IFN- γ responses. Further work on challenging issues related to process development for manufacturing and preclinical evaluation of HspC vaccine candidates for TB prevention is in progress. One recommendation is to mix HspCs with defined antigens, using former as an adjuvant.

Recent reviews on host defense against TB commonly do not consider humoral immunity. However, protective, non-protective and disease-enhancing mAbs to Mtb have been described. Consequently a vaccine that elicits protective antibodies might work. In other systems, IgM efficacy requires the right specificity and complement; IgG efficacy requires the right isotype and host. More antibody may not improve protection because of prozone-like effects. In the case of TB there is overwhelming evidence that antibody can contribute to host defense. However, the antibody response is complex and there are “good” and “bad” antibodies, the relationship between isotype, affinity and amount is not well understood and useful antibodies may be rare in the typical immune response to Mtb infection. Nevertheless, protection against Mtb with a vaccine that elicits antibody-mediated immunity is probably feasible. Possible mechanisms of action include opsonization, modulation of the inflammatory response, clearance of mycobacterial products and direct antibacterial effects.

Humoral immunity and TB: Vaccines inducing Antibody

Arturo Casadevall

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