Third International BCG and Immune Response Conference

October 7, 2017

Athens Greece

www.bcgandautoimmunity.org

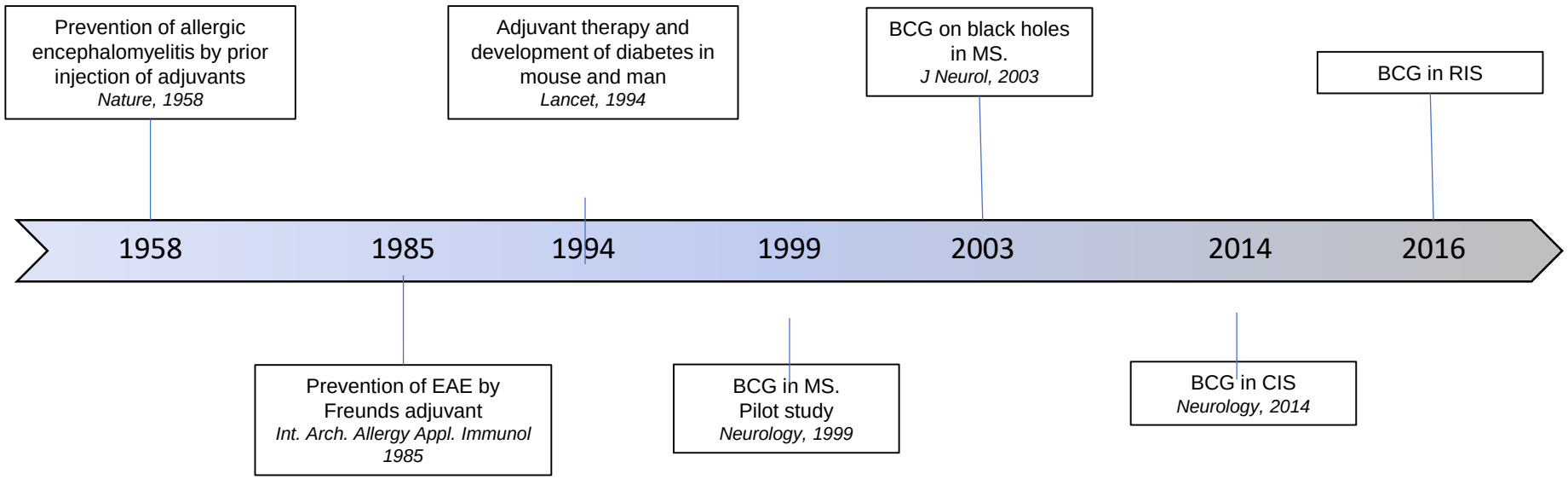
Agenda

- Barry Bloom, Harvard School of Public Health, United States: ***BCG, Tuberculosis & Autoimmunity***
- Giovani Ristori, Sapienza University of Rome, Italy: ***The BCG Story in Neuroinflammation***
- Nigel Curtis, University of Melbourne, Australia: ***The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR)***
- Denise Faustman, Massachusetts General Hospital, United States: ***BCG and Type 1 Diabetes***
- Christine Stabell Benn and Andreas Rieckmann, Statens Serum Institute, Denmark: ***Beneficial Non-specific Effects of BCG***
- Thomas Nørrelykke Nissen, Copenhagen University Hospital, Denmark: *Neonatal BCG Vaccinations*: ***The Danish Calmette Study***
- Graham Rook, University College London, United Kingdom: *The Old Friends Hypothesis*
- Godardhan Das, University of Kwazulu-Nata, India: ***Mycobacterium Tuberculosis Reprograms the Host Epigenome***
- Rob Arts, Netherlands, Radboud Medical Center, Holland: ***Remethylation of Genes***
- Marila Gennaro, Rutgers University, United States: ***Metabolic Reprogramming of Macrophages During Tuberculosis***
- Hazel Dockrell, London School of Hygiene & Tropical Medicine, United Kingdom: ***BCG Vaccination and Correlates of Protection***

BCG, Tuberculosis & Autoimmunity:
Barry R. Bloom

- BCG is the most widely used vaccine in the world, given to 104 million children a year and over 3 billion over time
  - The original BCG strain has been lost and thus there are many BCG strains used around the world with known genomic differences
  - BCG has had general high levels of protection against severe forms of tuberculosis (TB) in children, but the efficacy of BCG in preventing TB in adults has varied widely in different countries, varying from 77% in the UK to 0% in a large, 15-year trial in India
- BCG induces many T cell subsets, Th, Tregs, γδT cells, and many cytokines, including IFN-γ, TNFα, several interleukins and antibodies
  - While some of these cells and cytokines are known to be necessary for protection in humans, we do not know what combination is sufficient to provide protection and thus have no correlate or biomarker of protective immunity
- Latent infection with *M. tuberculosis* appears to provide better protection than BCG
- Among responses induced by *M. tuberculosis* and not effectively by BCG is the development of cytotoxic T cells (CTLs) that have the ability to kill both infected macrophages and intracellular bacilli.
- Tuberculosis infection in animals requires CTL for protection, whereas protection against BCG does not
  - The mechanisms by which BCG protects against autoimmune diseases remain mysterious and of interest

The BCG Story in Neuroinflammation:
Giovanni Ristori



BCG vaccine proven to reduce disease activity and tissue damage in early multiple sclerosis

A trial to prevent or delay disease onset in people with radiologically isolated syndrome (RIS) is in progress

The Melbourne Infant Study, BCG for Allergy and Infection Reduction:
Nigel Curtis

- A randomized controlled study of BCG vaccination shortly after birth in Melbourne, Australia
  - Over 1200 newborns being followed up to 5-years of age
- Extensive detailed antenatal, perinatal and postnatal data
  - Collected by parent-completed online questionnaires
  - Clinical outcome measures: Respiratory infections, eczema, allergic sensitisation (measured by skin prick test) and asthma
  - Large biosample collection to understand molecular and immunological mechanisms of BCG: Plasma, PBMC, RNA, DNA, stool and respiratory samples
- There are differences between BCG vaccines strains, the same strain made by different manufacturers, and also between batches made by individual manufacturers
  - Further, the impact of the differing CFU concentrations between BCG vaccines strains is unknown and largely ignored; e.g. BCG Russia contains up to 10-fold more CFU/ml than BCG Tokyo
  - Different BCG vaccine strains induce a different immune response and have different protective efficacy against TB
  - A study in Kazakhstan found that for protection against TB, BCG-Japan > BCG-Serbia > BCG-Russia
  - A large randomised trial in 303,092 neonates in Hong Kong found that the risk of TB with BCG-Pasteur vaccine was 45% (95% CI 22% - 61%) less than with BCG-Glaxo
  - The non-specific effects of BCG vaccine also differ between genotypes and manufacturers

BGC and Type 1 Diabetes:
Denise Faustman

- Type 1 diabetes is an autoimmune disease cause by autoreactive T-cells attacking the islets in the pancreas and too few functional suppressive cells (regulatory T cells, or Tregs)
  - BCG has been hypothesized as a potential method to reverse type 1 diabetes
  - BCG induces TNF, a cytokine proven to kill autoreactive T cells and induce Tregs
  - BCG appears to create a host relationship with the immune system that induces the proliferation of Tregs
  - The strain, dose and timing of vaccination is important
  - New data from all groups working on BCG in autoimmunity suggest the duration of the trials need to be at least 3-4 years to see the clinically meaningful effects
  - New data suggests BCG might be clinically meaningful even if used in long-term type 1 diabetic subjects
  - Early studies dosing BCG in new onset type 1 diabetes were inconclusive possibly because of use of a BCG strain that is not effective in autoimmunity, the dosing used and no-long term follow up
  - New data from ongoing type 1 trials show a significant response in type 1 diabetes and lasting changes in Treg populations
- A Phase II FDA approved clinical trial of 150 patients with existing type 1 (not prevention or new onset) is underway - More info at www.faustmanlab.org

Beneficial Non-Specific Effects of BCG:
Christine Stabell Benn and Andreas Rieckmann

- BCG is associated with substantial mortality reductions among children in low-income countries
  - BCG reduces child mortality by at least a third, far more than can be explained by prevention of tuberculosis and, like other live vaccines, BCG has beneficial *non-specific effects*
  - In Denmark, BCG at school age was also associated with strong mortality reductions into early adulthood
  - In Guinea-Bissau and Denmark, the development of BCG scars is highly dependent on vaccination technique; thus, BCG scar development may be used as a proxy for successful BCG vaccination and is associated with strong mortality reductions
  - BCG had a particularly beneficial effect in children whose mothers were BCG vaccinated, both in Guinea-Bissau and in Denmark
    - Maternal priming may be important in order to elicit the beneficial non-specific effects of BCG on morbidity and mortality
  - Revaccination ("boosting") with BCG has also been associated with amplification of its beneficial non-specific effects on morbidity and mortality
  - The beneficial effects of priming and boosting have also been seen for other live vaccines
    - We may be designed to benefit from meeting pathogens in the presence of pre-existing immunity, be it from our mother or from previous exposure

Neonatal BCG Vaccinations: The Danish Calmette Study:
Thomas Nørrelykke Nissen

Clinical outcomes of neonatal BCG vaccinations:

- No overall effect on hospitalisations
- Mild beneficial effect on GP visits from 0-3 months of age (12%)
- Mild beneficial effect on atopic dermatitis (10%); stronger in predisposed children (16%)
- Effects more pronounced in children of mothers who were BCG-vaccinated –eg. infection hospitalizations (35%)
- Effects were stronger in the first 3 months of life - before other vaccines were given – as also seen elsewhere

Immunological outcomes:

- No overall effect on immunological outcomes
- Consistent effect modification of BCG by age of vaccination
- Poor BCG-specific IFN-γ response

The Old Friends Hypothesis:
Graham Rook

T1D and the other associated autoimmune states are at least partly attributable to environmental change leading to defective immunoregulation

- Disorders of immunoregulation are increasing in high-income urban settings (i.e., autoimmunity, allergies & inflammatory bowel diseases, as well as metabolic and psychiatric disorders linked to failure to terminate unwanted inflammation)
- At birth, the immune system requires “data” inputs from microorganisms with which humans co-evolved, including the symbiotic microbiota (particularly in the gut) and organisms from the natural environment that modulate development of organ systems
- Perinatal antibiotics, caesarean deliveries, lack of breast-feeding, bad diet, and lack of exposure to the natural environment all lead to defective microbial inputs, distorted microbiota and poor immunoregulation
- Poor immunoregulation increases the inflammatory response to perinatal psychosocial stressors, which further increase the risk of T1D, celiac disease & multiple sclerosis.
- Mycobacteria are abundant in the natural environment, and humans probably evolved with subclinical mycobacterial infections that could persist in human tissues
- Mycobacteria drive cytokine release and multiple immunoregulatory pathways
- Multiple BCG vaccinations might replace such mycobacterial infections or compensate for other defective microbial inputs, and so promote immunoregulation

Mycobacterium Tuberculosis Reprograms the Host Epigenome:

Gobardhan Das

Active TB

- BCG is the only usable vaccine available for TB, but its efficacy is less than satisfactory
- Optimum vaccine efficacy requires both Th1 and Th17 responses in the lung, but BCG predominantly induces only Th1 responses in the lung
- Virulent *M.tb* modulation of immune responses in lung occurs by epigenetic modification: *M.tb* regulates miR146a in macrophages which in turn controls IL-6 production in infected macrophages
    - IL-6 is the key cytokine required for the differentiation of Th17
- Furthermore, BCG is unable to mount the threshold of central memory T cells (Tcm) for long lasting host protection
- Blocking of Kv1.3, a potassium channel predominantly expressed by effector memory T (Tem) cells, while vaccination with BCG dramatically enhances Tcm
- Enhanced Tcms in BCG vaccinated animals improves vaccine efficacy by 50-100 fold

Latent TB

- Mesenchymal stem cells (MSCs) provide niche for dormant TB, where TB bugs hide for an extended period in an inert condition and are non-responsive to antibiotics
- *M.tb* enters MSCs using receptors and survives within cytosol
- In the cytosol, *M.tb* induces lipid synthesis and thereafter hides within lipid droplets in the cytosol, and the lipid is used as a carbon source
- *M.tb* modulates different sets of miRNA in macrophages for survival within MSCs in a dormant state

BCG-Induced Epigenetic Histone Changes:
Rob Arts

- BCG has non-specific effects and protects from other infections
  - SCID mice (which lack adaptive immunity) are protected from lethal candidiasis after BCG vaccination
    - Hence the non-specific effect of BCG is at least partially mediated by the innate immune system
  - BCG induces trained immunity in monocytes in humans, which means that they produce more cytokines upon restimulation with non-related pathogens
  - Trained immunity induces epigenetic histone changes (H3K4me3 and H3K27ac) in human monocytes, both *in vitro* and *in vivo*
  - Induction of cytokine production by BCG is correlated with induction of epigenetic changes

Metabolic Reprogramming of Macrophages During Tuberculosis: Marila Gennaro

- *Mycobacterium tuberculosis* is an intracellular pathogen that parasitizes host macrophages
- Infected macrophages show induction of aerobic glycolysis, downregulation of oxidative phosphorylation, and accumulation of neutral lipids
- Macrophage metabolic changes often result in impaired innate immune responses and facilitate mycobacterial persistence
- Understanding the mechanisms of macrophage metabolic reprogramming identifies targets for host-directed therapy against tuberculosis
- Host-directed therapy may help prevent or overcome the appearance of antibiotic resistance

BCG Vaccination and Correlates of Protection:
Hazel Dockrell

- We have immunological assays that measure the immunogenicity of BCG vaccination (e.g., using IFNg production), but there are as yet no confirmed correlates of protection
  - Mycobacterial growth inhibition assays look promising
- Epigenetic changes associated with response to BCG:
  - DNA methylation patterns are different in IFNg high or low responder South African infants tested 10 weeks after BCG vaccination
  - Pathway analysis shows metabolic and cell function pathways are involved as well as immune response genes
- Non-specific immunity induced by BCG vaccination:
  - Enhanced cytokine responses to non-specific stimuli can be detected in peripheral blood cells from BCG vaccinated UK infants; changes in NK cell numbers are also seen
- Other factors that may contribute to variable responses to BCG:
  - Maternal exposure to mycobacterial antigens through BCG vaccination or latent TB infection may influence infant BCG vaccination responses, although some changes are transient
  - Other coinfections (i.e., intestinal helminths) or comorbidities (i.e., type 2 diabetes) can affect immune responses, mycobacterial growth inhibition and gene expression signatures.