Tuberculosis: Here today...... and here tomorrow.

David G. Russell

Infected mouse lung: actin (blue), nuclei (gray), Mtb (red), lipid inclusions (green)
Tuberculosis Eradication

1919

28th October, 2015
Tuberculosis is predominantly a pulmonary infection.

1. The bacterium is inhaled and sets up the infection in macrophages in the lung.

2. In most individuals this “granuloma” contains the infection.

3. TB-positive individuals have a 5-15% likelihood of developing active disease over their lifespan.

4. This likelihood is increased to 5-15% per years if co-infected with HIV.

Three Short Perspectives.

1. The concept of immune control (Vaccines).

2. Drug discovery within the context of the host environment (Drugs).

1. The co-operativity between HIV and tuberculosis infections (HIV).
A Depressingly Familiar Headline
(the MVA85A vaccine, 2013)

We have allowed ourselves to become locked into an immunological paradigm for “protection”.

New TB Vaccine Fails Trial
One of the most advanced tuberculosis vaccines has failed to protect infants from getting the disease in a clinical trial, but it may be effective in adults.
By Dan Cossins | February 4, 2013
All Existing Vaccine Strategies Emphasize Control as a Desirable Gain-of-Function

In Humans:

Because we cannot do live challenge, vaccine “success” is measured by the induction of peripheral immune correlates thought to correspond to protective immunity.

- Cytokine production
- T-cell markers
- Immune recognition
Controller versus Permissive Phagocytes?

Controller Cells
- Th1 activated macrophages
- Product of IFN-γ exposure
- Good at controlling Mtb in vitro and in vivo (in mice)
- Efficacy revealed in loss of function studies (KO mice)

Permissive Cells
- M2 macrophages or another cell type?
- Exposure to IL10 or TGF-β?
- Permissive for bacterial expansion in vitro and in vivo?
- We have minimal data on bacterial permissiveness as an immune function.

- Vaccine efficacy assessed by immune correlates of Th1

- We need bacterial correlates of fitness not immune correlates.

What if disease progression is mediated by an expansion of permissive host cells and NOT a loss of control? This is not a semantic argument if permissiveness is a real, gain of function phenotype.
Mince, digest with collagenase, pass through a tissue strainer to generate a single cell suspension

Live sort for Mtb mCherry infected phagocytes to explore the phenotype of the phagocyte populations in the mouse lung

Strategy for the Functional Dissection of the Phagocytes in the Infected Mouse Lung

Lu Huang
Mtb is differentially distributed in Alveolar Macrophages, Interstitial Macrophages and Dendritic Cells.

We can sort on those cells that are infected with mCherry-expressing Mtb.

The distribution is extremely plastic in the first 6 weeks of infection.
Utilization of Mtb reporter strains to map bacterial fitness. The % Foci-Positive Bacilli is inversely proportional to the Immune Response.

Proposed Studies to Pursue In Vivo Phenotype in both non-human primates and active human infections

Continue to use the mouse to refine our methods and to define those cells types of greatest interest as permissive hosts

JoAnne Flynn, Pittsburgh
Perform comparable reporter bacteria experiments on experimental NHP infections to validate cell phenotype in an accessible primate model

Henry Mwandumba, MLW
Perform Mtb growth/fitness studies on airway macrophages from uninfected and Mtb-infected individuals

The Long-term Goal is to Develop a Strategy to Reduce the Incidence or Induction of Permissive Host Cells
2. Drug Discovery within the context of the host cell environment

- Reactive nitrogen intermediates
- Acquisition of lysosomal hydrolases
- Acidic pH (pH 6.4)
- Superoxide burst
- Nutritional restriction
High-throughput Screen against intracellular Mtb (mCherry)

J774 cells
mCherry Mtb
384 well plates
6 day incubation

340,000 compounds

Christopher Locher and Christine Memmott
Vertex Pharmaceuticals
Summary of Validated Compounds

340,000 compounds

2400 hits from primary screen: 300 had IC50 < 5 µM

144 compounds equally potent in cells and in broth (includes known drugs)

16 compounds show increased potency in broth

147 compounds show markedly higher potency inside cells

Nutritional restrictions within a host cell

Cholesterol
Fatty acids

Glycerol
Glucose

Carbohydrates
Nucleotides

Host lipids

G6P
F1,6P
PEP
host cholesterol

PEPCK

Acetyl-CoA
Oxaloacetate
Isocitrate

Malate
Succinate

Prop-CoA

ICL
ICL

Succinate
Malate
Oxaloacetate
Acetyl-CoA

carbohydrates nucleotides
Carbon source influences compound activity *in vitro*

101 conditionally-active compounds tested*

- 40 compounds in cholesterol media with IC50 < 5.0 μM
- 33 compounds in acetate media with IC50 < 5.0 μM
- 2 compounds in both media with IC50 < 5.0 μM

*readout: alamar blue mCherry signal

33 compounds IC50 < 5.0 μM in 7H12 (minimal medium) with cholesterol or acetate
New Cholesterol-Dependent Inhibitors.

340,000 compounds

Uptake apparatus

mce4

cholesterol

Metabolic Regulation

Sterol ring breakdown

Propionate detoxification

Cholesterol-dependent cell wall synthesis

M.tb central metabolism

Cell Wall Synthesis

priony-CoA

PrpC

HsaAB

sterol synthesis

340,000 compounds
Cholesterol constrains the metabolism of Mtb in the host

Mouse Mo with mCherry Mtb & Labeled cholesterol (green)

Host cholesterol + sugars + inhibitor

In the presence of cholesterol the bacterium is unable to co-metabolize other carbon sources (Carbon Catabolite Repression).
Tuberculosis is now the leading single killer of individuals living with HIV infection.

Impact of HIV infection on lung immune function

Queen Elizabeth Hospital Blantyre, Malawi

Work with Henry Mwandumba and Kondwani Jambo
The Make-up of the Cellular Populations in the Lung

The cells in the lung airways are approximately 75% macrophages and 25% lymphocytes. This is not altered by asymptomatic HIV infection.
Validation of the FISH detection platform

Hela Cells infected with varying doses of pseudotyped NL4-3 BaL (env)::GFP HIV.

The FISH label for gag mRNA correlates with GFP expression, localizes to the same cell population, and is ablated by treatment of the cells with RNaseA.
HIV is preferentially located in the alveolar macrophages.

In all individuals examined the % abundance of HIV-infected cells is higher in the macrophage population than it is in the lymphocyte population.

Alveolar Macrophages: Facts to remember

1. Alveolar macrophages are a self-sustaining tissue-resident population independent of peripheral blood monocytes (Gomez Perdiguero et al. Nature. 2015).

2. In mice, alveolar macrophages can survive the life-span of the animal, effectively in excess of one year (Murphy et al AJRCMB. 2008).

3. Human alveolar macrophages show elevated levels of expression of the pro-survival genes (Flaherty et al JBC. 2006).

4. And..... macrophages are not killed by HIV infection.
Combination Anti-Retroviral Therapy (cART)

Current frontline therapy against HIV consists of a cocktail of 3 reverse transcriptase inhibitors.

Hits a single stage of the viral life cycle that blocks NEW infection.

But has NO impact on existing HIV infections.

The success of ART is dependent on HIV being cytotoxic and inducing Immune-mediated clearance.

ART does not kill infected cells.
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Christine Memmott,
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Henry Mwandumba and Kondwani Jambo

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