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Medical Microbiology and Hygiene  
University Hospital Ulm  
MyTB Lab  

Fight against Tuberculosis:  
General Aspects
Physical barrier of the respiratory epithelium

cilia cells
(with tiny hairs to move mucus out of your lungs)
mucus cells
(make mucus)

mucus
(released from cell to trap dirt particles)

Airway Walls

(idw)
Mycobacteria (may) pass the upper airways and reach the alveolar space.
.....where they interact with pattern recognition receptors of macrophages and dendritic cells
The physical barrier of the upper airways and innate immune cells eliminate the majority of bacilli

Index case

5 close contacts
( >8hrs in a closed room)

50-80%: no infection (IGRA-negative)

20-50%: infection (IGRA conversion)

2-10%: active disease

90-98%: latent infection

5% lifetime risk for reactivation
Immunity to *Mycobacterium tuberculosis*

antimicrobial activity

nitric oxide cathelicidin autophagy
PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO-release: An antibiotic mimics immunity.
Pretonamid (PA-824) as a therapy against tuberculosis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Sponsor(s)</th>
<th>Phase</th>
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- QT-prolongation, liver toxicity (3 fatalities)

http://www.tbonline.info/medicines/
Toll-like receptors sense *Mycobacterium tuberculosis* and induce innate and acquired immunity.

Stenger et al., *Curr. Opin. Immunol.*, 14: 452, 2002
TLR2/1 ligands induce antimicrobial activity in macrophages

Liu et. al., Science, 291, 2001
TLR2/1 ligands induce antimicrobial activity in macrophages, but not dendritic cells.

BAD NEWS CAN BE GOOD NEWS (FOR SMART SCIENTISTS)
Search for immunomodulatory genes that are selectively induced in macrophages

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<td>TLR2/1L</td>
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<td>dendritic cells</td>
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<tr>
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<tr>
<td>TLR2/1L</td>
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- Vitamin D receptor (A)
- Vitamin D receptor (B)
- S100A12
Vitamin D and tuberculosis

**VDR polymorphisms may influence susceptibility to mycobacterial disease**


VDR polymorphisms may influence susceptibility to mycobacterial disease

Tuberculosis patients have lower VitD serum levels than healthy contacts
(Sita-Lumsden et al., Thorax, May 2007)

Vit D3 induces the oxidative burst and enhances phagolysosome fusion in *M. Tb*-infected macrophages

Vit D3 enhances antimycobacterial activity of monocytes and macrophages
(Rook et al., Immunology, 57: 159, 1986; Crowle et al., Infect. Immun., 55: 2945, 1987; Denis et
Cathelicidin

The promoter of the human cathelicidin peptide has a **VitD response element** (Wang et al., J. Immunol., 173: 2909, 2004)

Expression in **macrophages, lymphocytes, neutrophils and epithelial cells** (Wah et al., Cell Tissue Res., 324: 449, 2006)

siCath specifically knocks down VitD mediated antimicrobial activity

$^{3}$H-uracil (% control)

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<thead>
<tr>
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<th>control</th>
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<tr>
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<tr>
<td>no siRNA</td>
<td>*</td>
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<tr>
<td>siCtrl</td>
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* p<0.05

CFU (% control)

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<th>1,25D3</th>
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<td>siCtrl</td>
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</tr>
<tr>
<td>no siRNA</td>
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</tbody>
</table>

* p<0.05
**Conclusion**

- **TLR2** and **TLR4** ligands
- Vitamin D (VitD)
- Vitamin D receptor (VDR-RE)
- Genes
- Cathelicidin (LL-37)
- Pro-VitD
- Vitamin D receptor
- Retinoid X receptor

Vitamin D-mediated antimicrobial activity is dependent on Cathelicidin and Autophagy.
Autophagy Is a Defense Mechanism Inhibiting BCG and *Mycobacterium tuberculosis* Survival in Infected Macrophages

Maximiliano G. Gutierrez,¹,² Sharon S. Master,² Sudha B. Singh,² Gregory A. Taylor,³ Maria I. Colombo,¹,* and Vojo Deretic²,4,*
Immunological Factors that induce Autophagy

Espert et al., Front Cell Infect Microbiol, 5, 2015
Pharmacological Agents that Target Autophagy

Evaluation of vitamin D as therapy against tuberculosis

Basic finding: Vit D supports antimycobacterial activity of human macrophages

Advantage: well tolerated, available as an oral drug, cheap

Next step: clinical evaluation
Vitamin D as supplementary treatment for tuberculosis
(Wejse et al., Am J Respir Crit Care Med, 179: 843, 2009)

- double blind, randomized, placebo controlled trial
- 365 tuberculosis patients, 281 completed follow up
- Vitamin D intervention: 100,000 IU at months 0, 5, 8
- no significant differences in side effects
High doses of vitamin D₃ fail to reduce the time to sputum culture conversion

Vitamin D₃: 36 days
Placebo: 43.5 days

$p=0.41$ (long-rank test)

Martineau et al., Lancet, 377: 242, 2011
Vitamin D as supplementary treatment for tuberculosis
(Wejse et al., Am J Respir Crit Care Med, 179: 843, 2009)

- double blind, randomized, placebo controlled trial
- 365 tuberculosis patients, 281 completed follow up
- Vitamin D intervention: 100,000 IU at months 0, 5, 8
- no significant differences in
  side effects
  sputum conversion
  outcome (mortality 15%)
High dose- VitD3 reduces the time to sputum culture conversion in patients with TaqI genotype of the VDR

<table>
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<tr>
<th>VDR genotype</th>
<th>p-value</th>
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<tr>
<td>TaqI</td>
<td>0.03</td>
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<tr>
<td>tt</td>
<td>0.02</td>
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<td>Tt</td>
<td>0.63</td>
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<td>TT</td>
<td>0.71</td>
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<tr>
<td>Fokl</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Vitamin D as Adjunctive Host Directed Therapy

Wallis and Zumla, Open Forum Infectious Diseases, DOI10:1093/ofid/ofw151
Vitamin D and Phenylbutyrate as Host Directed Therapy

Wallis and Zumla, Open Forum Infectious Diseases, DOI:10.1093/ofid/ofw151
Hermann Brehmer (1826-1889)

Biology student, that was cured from tuberculosis in the Himalaya region.

received his PhD for a thesis on „Tuberkulose ist eine heilbare Erkrankung“

Founded the world´s first sanatorium in 1854 in Silesia
Table 1 | Results as shown in 1848 study

<table>
<thead>
<tr>
<th></th>
<th>Standard treatment</th>
<th>Standard treatment plus cod liver oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>542</td>
<td>535</td>
</tr>
<tr>
<td>Improved</td>
<td>60.8%</td>
<td>63.1%</td>
</tr>
<tr>
<td>Arrested</td>
<td>5.6%</td>
<td>18.1%</td>
</tr>
<tr>
<td>Deteriorated or died</td>
<td>33.3%</td>
<td>18.8%</td>
</tr>
</tbody>
</table>

The first Nobel Prize for tuberculosis research goes to ............
The first Nobel Prize for tuberculosis research goes to.............
Niels Ryberg Finsen (1860-1904)

1903: Nobel Prize for the introduction of UV therapy in the treatment for tuberculosis of the skin (Lupus vulgaris)
*M. tuberculosis* prevents acidification of lysosomes
Figure 1  PknG affects the intracellular traffic of *M. tuberculosis* in macrophages. Most microbes and nonpathogenic mycobacteria quickly find themselves in lysosomes, where they are killed. By contrast, *M. tuberculosis* stays within phagosomes; the bacterium releases PknG to block phagosome-lysosome fusion. Bacteria lacking pknG are rapidly transferred to lysosomes and eliminated. Modified from ref. 20.

Walburger et al., Science, 304: 1800, 2004
M. tuberculosis prevents acidification of lysosomes

Therapeutic intervention: overcome acidification block
Example: interferon-γ (MacMicking, Russell, Deretic)
Problem: intolerable toxicity upon systemic administration
Search for small molecules capable of overcoming the *M. tuberculosis*-mediated maturation block
Abl tyrosine kinase: Role in immunity

- T cell development, signalling, proliferation, CD8+ T cell expansion and cytokine production

- maturation of monocytes to dendritic cells

- modulation of autophagy

- modulation of trafficking and function of lysosomes

1 Gu et al., Immunol. Rev., 228: 170, 2009
2 Appel et al., Blood, 103: 538, 2004
Pharmacological inhibition of Abl tyrosine kinase limits the growth of intracellular *M. tuberculosis*.

Bruns et al., J Immunol, 189: 4069, 2012
Imatinib-Sensitive Tyrosine Kinases Regulate Mycobacterial Pathogenesis and Represent Therapeutic Targets against Tuberculosis

Ruth J. Napier,1 Wasiulla Rafi,5,6,7 Mani Cheruvu,3,7 Kimberly R. Powell,2 M. Analise Zaunbrecher,1,3 William Bommann,4 Padmini Salgame,6 Thomas M. Shinnick,3 and Daniel Kalman2,* Napier et al., Cell Host & Microbe, 10: 475, 2011

Figure 7. Imatinib Reduces Bacterial Load in Mice Infected with Mycobacterium tuberculosis

(A) C57Bl/6 mice were infected with 50–100 cfu of aerosolized Mtb Erdman. Beginning 24 h prior to infection, animals were administered water (carrier) or imatinib at concentrations of 66.7 mg/kg/day or 100 mg/kg/day. Cfu was determined in right superior lobe of the lung at 28 days p.i. Solid lines represent the median cfu; dotted line represents the limit of detection (10 cfu); p values were determined by a nonparametric Kruskal-Wallis test.

(B) C57Bl/6 mice were administered carrier pumps 24 h prior to infection. Unmanipulated or carrier-treated mice were infected with a low (2.5 × 10^5 cfu; left) or high dose (1 × 10^6 cfu; right) of aerosolized Mtb Erdman and cfu determined in the whole lung at 24 h p.i. The solid line represents the median cfu.

(C and D) C57Bl/6 mice were infected with 2.5 × 10^5 cfu of aerosolized Mtb Erdman. Beginning 24 h prior to infection, animals were either left untreated or administered carrier (water) or imatinib at a concentration of 66.7 mg/kg/day. Cfu was determined by plating homogenates of the whole lung (C) or spleen (D) at 28 days p.i. The solid line represents the median cfu; dotted line represents the limit of detection (10 cfu); p values were determined by a nonparametric Mann-Whitney test.

Delivery of Drugs In Vivo
Innate immunity alone fails to prevent infection with *Mycobacterium tuberculosis* in 20-50% of the cases.

Index case

- 5 close contacts (>8hrs in a closed room)

  - 50-80%: no infection (IGRA-negative)
  - 20-50%: infection (IGRA conversion)
    - 2-10%: active disease
    - 90-98%: latent infection
      - 5% lifetime risk for reactivation
Adaptive immunity to *Mycobacterium tuberculosis*

- **T-cell-recruitment**
  - CCL-2, CCL3, CCL-5, TNF

- **antigen-specific T-cell-activation**
  - IL-12, IL-18, IL-23
  - IL-7, IL-15, IL-21
  - Th1-response
  - Th17-response
  - Cytotoxic T cells

- **antimicrobial activity**
  - Nitric oxide
  - Cathelicidin
  - Autophagy

- **regulatory T-cells**
  - IL-10, TGF-β
Major T cell subsets in human tuberculosis

Kaufmann et al., Int J Tuberc Lung Dis, 10: 1068, 2006
CD8+ cytolytic T cell

CD4+ T helper cell

TCR

CD3

lipid or lipoglycan

peptide

MHC molecules

Fc receptors
complement receptors
scavenger receptors

TLR 2/4
DC-SIGN

innate immunity

granuloma

IFN-γ

TNF

tn

MHC molecules

peptide

TCR

CD3

CD1

CD4+ T helper cell
CD8+ cytolytic T cell

acquired immunity

effector (memory) cells

IL-2

TNF
Natural course of infection with Mycobacterium tuberculosis

- 5%: active TB
- 95%: latent TB
Treatment of autoinflammatory diseases with TNF antibodies

- Infection
- Rheumatoid arthritis
- Anti TNF therapy

5%: active TB
95%: latent TB
CD8+ cytolytic T cell

CD4+ T helper cell

TCR

CD3

lipid or lipoglycan

peptide

MHC molecules

Fc receptors
complement receptors
scavenger receptors
TLR 2/4
DC-SIGN

innate immunity

acquired immunity

granuloma

IFN-γ

TNF

TNF

CD4+ T helper cell

CD8+ cytolytic T cell

effect (memory) cells

IL-2

TNF

DC-SIGN

TLR 2/4

complement receptors

Fc receptors

scavenger receptors

CD1
**Innate immunity**

- CD8+ cytolytic T cell
- CD4+ T helper cell
- Effector cells

- Lipid or lipoglycan
- Peptide
- MHC molecules
- Fc receptors
- Complement receptors
- Scavenger receptors
- TLR 2/4
- DC-SIGN

**Acquired immunity**

- CD4+ T helper cell
- CD8+ cytolytic T cell

- Effector cells
Reactivation of tuberculosis during Infliximab therapy

5%: active TB
95%: latent TB
From Basic Immunology to an efficient Vaccine (?)
Available Vaccines for Humans

- rubella
- measles
- mumps
- chicken pox
- influenza
- Hepatitis A
- Hepatitis B
- rabies
- polio
- yellow fever
- tick-borne encephalitis
- japanese encephalitis
- rotavirus
- papilloma virus

viral diseases:
protection by neutralizing antibodies
## Available Vaccines against Bacterial Infections

<table>
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<tr>
<th>toxoid</th>
<th>polysaccharide/protein conjugate</th>
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<tr>
<td>- tetanus</td>
<td>- meningococci</td>
</tr>
<tr>
<td>- diphtheria</td>
<td>- pneumococci</td>
</tr>
<tr>
<td>- pertussis</td>
<td>- Haemophilus influenza B</td>
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<tr>
<td></td>
<td>- typhoid fever</td>
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Protection based on neutralizing antibodies directed against toxins or the bacterial capsule.
**Innate Immunity**
- Fc receptors
- Complement receptors
- Scavenger receptors
- TLR 2/4
- DC-SIGN

**Acquired Immunity**
- CD4+ T helper cell
- CD8+ cytolytic T cell
- MHC molecules
- TCR
- Lipid or lipoglycan
- Peptide

**Granuloma**
- IFN-γ
- TNF

**Effector (Memory) Cells**
- IL-2
- TNF

**Effector Cells**
- DC (Dendritic Cell)
- Effector (memory) cells

**IFN-γ**
- Interferon-γ

**TNF**
- Tumor necrosis factor
Tuberculosis: The Ideal Vaccine
Prevention of Tuberculosis: BCG Vaccination

- 1900: Institute Pasteur in Lille, Albert Calmette / Camille Guerin

- subculture in ox bile reduced virulence in guinea pigs

- 1902: strain from a tuberculous cow provided by Nocard

- 1908: subculture of the strain from 1902 in 3-week intervals

- 1913: initiation of a vaccine trial in cattle

Luca and Mihaescu, Maedica, 8: 53, 2013
Figure 5.1.1 Comparison of protective efficacy of live BCG with that of other vaccine preparations in cattle
The Lübeck Accident

251 infants were BCG (provided by Guerin) vaccinated in 1929 and 1930

72 (29%) died of tuberculosis within 2-5 months
135 (54%) developed tuberculosis, but recovered
44 (17%) became tuberculin-positive, but remained well

1931/1932:
Prof. Georg Deycke, Dr. Ernst Altstaedt and Anna Schütze were convicted for manslaughter
Prevention of Tuberculosis: BCG Vaccination

1930: increasing criticism against Calmette and Guerin even though BCG was not directly responsible for the „Lübecker Impfungleck“
Prevention of Tuberculosis: BCG Vaccination

- 1930: increasing criticism against Calmette and Guerin even though BCG was not directly responsible for the „Lübecker Impfunglück“

- 1940s: spread and propagation of BCG by UNICEF, WHO

- 1950s: major clinical trials in United Kingdom (Copenhagen strain, successful) and United States (Tice strain, unsuccessful)

- Most countries in the world implemented BCG vaccination

- Most widely used vaccine worldwide (4 billion doses)
BCG-Vaccination: Efficacy

Haiti 1973: 80% protection
Great Britain 1977: 75% protection
Chingleput 1980: no protection
Georgia, Alabama 1969: no protection
Northern Malawi 1992: no protection

Protection from severe disease in childhood, but not from infectious pulmonary tuberculosis
Reasons for Variable Efficacy of BCG

- variable exposure to environmental mycobacteria
- co-infections (helminths)
- nutritional status (iron, vitamin D)
- ethnicity, genetic background, immune status (HIV!)
- BCG is not clonal: different strains at different sites
- different quality of BCG (viability per dose)
BCG: Advantages

1. protection against severe disease in children
2. generally well-tolerated
3. practical: single dose to newborns
BCG vaccination policy by country

A. The country currently has a universal BCG vaccination programme.
B. The country used to recommend BCG vaccination for everyone, but currently does not.
C. The country recommends BCG vaccination only for specific groups.


* BCG = Bacille-Calmette-Guérin

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Vaccine approaches: Examples

1. Purified lipid antigens
2. Genetically modified *M. smegmatis* ("IKEPLUS")
3. Genetically modified *BCG* (rBCGUreC:Hly, Aeras-422)
Urease-deficient BCG expressing listeriolysin

Figure 2. Schematic description of the underlying mechanism of improved T cell stimulation by a novel BCG vaccine. Recombinant BCG deleted in urease and expressing listeriolysin is capable of inducing a more profound immune response than wild-type BCG. The likely mechanism involves perforation of the phagosomal membrane, which allows leakage into the cytosol of both mycobacterial antigens and phagosomal enzymes such as cathepsins. Cathepsins are known to induce apoptosis. Thus, the new BCG vaccine strain induces crosspriming leading to a more efficacious immune response. Image provided by Stefan Kaufmann (Berlin, Germany) (25).
Vaccine approaches: Examples

1. Purified lipid antigens (Ac$_2$SGL)
2. Genetically modified *M. smegmatis* („IKEPLUS“)
3. Genetically modified *BCG* (rBCGUreC:Hly, Aeras-422)
4. Genetically modified *M. tuberculosis* (PhoP mutant)
5. Proteins expressed in viral vectors (MVA85A)
Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Michele D Tameris*, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hassan Mahomed†, Helen McShane†, and the MVA85A O20 Trial Study Team

Lancet, 381: 1021, 2013
Vaccine approaches: Examples

1. Purified lipid antigens (Ac$_2$SGL)
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3. Genetically modified *BCG* (rBCGUreC:Hly, Aeras-422)
4. Genetically modified *M. tuberculosis* (PhoP mutant)
5. Proteins expressed in viral vectors (MVA85A)
6. Fusion proteins in adjuvant (H56 in IC31)
Prevention of *M. tuberculosis* Infection with H4:IC31 Vaccine or BCG Revaccination


Phase 2b Controlled Trial of M72/AS01E Vaccine to Prevent Tuberculosis

Towards an improved tuberculosis vaccine: Perspective

- at present the problem is not the availability of vaccine candidates

- bottle neck: number of vaccines that can be tested in clinical efficacy trials given the limited clinical trial capacity worldwide

- desperately needed: surrogate endpoint markers as correlates of protection to reduce the need for long term, large scale clinical trials

- development of predictive animal models

- establish improved routes of delivery e.g. aerosol