Diabetes and Tuberculosis (DM/TB)
Synergistic Epidemics and Pathogenesis Mechanisms

TCRB, May 2016
A Disease on the Rise


http://www.nytimes.com/2014/04/26/opinion/sunday/the-global-diabetes-epidemic.html?_r=0

By KASIA LIPSKA  APRIL 25, 2014
Converging Epidemics

• **Globally**, DM incidence will increase **30%+** by 2035
  – 592 million cases
• 2013 – 15% of TB/DM cases – 10^6
• Countries with highest TB burden
  – incidence rates of TB/DM
    2009 – 10%
    2013 – 15% - 50% increase
• **Mexico** – 2000-2012 → TB/DM cases **increased** 83%
  while TB cases without DM **decreased** 27%
• **India** – DM/TB case incidence
  1998 - 2006 → 900,000 new TB cases attributable to DM,
  essentially **negating** the effects of improved TB control
• How does DM affect the risk of developing active TB and treatment outcomes?
Key finding in the Systematic Review

Diabetes increases the **risk of active TB** about **3-fold**, risk seems to increase with poor glucose control.

Diabetes increases the risk of adverse TB treatment outcomes - Meta-analysis studies (#)

- Higher **relapse rate** \( RR = 3.9 \) (5)
- Higher **death rate** \( RR = 1.9 \) (23), but \( RR = 4.9 \) in the 4 studies with adjustments for age and other confounders

**No trials** on the efficacy of alternative TB treatment regimens in people with diabetes.
• What are the dual DM-TB pathogenic mechanisms and are they synergistic?
Pathogenesis of DM complications

DM is also an inflammatory disease “Metaflammation” (Hotamisligil)

Beginning with simple chemical reactions:

• GENERATION OF “glycation” molecules
  – Increased pro-inflammatory signaling RECEPTORS and disruption of cell regulatory pathways

• Glycated/oxidized lipids
  – Uptake by scavenger receptors, LOX-1, CD36 – lipid accumulation, M2 macrophage polarization
carboxyethyllysine (CEL), N’-carboxymethyllysine (CML), 3-deoxyglucosone-derived lysine dimer (DOLD), methylglyoxal lysine dimer (MOLD), glyoxal-derived lysine dimer (GOLD)
RAGE is a primary receptor for AGEs, but AGEs also interact with macrophage TLRs, NLR’s.

**Hyperinflammatory state** –

- Increases MAPK/NF-κB signaling and cytokines
- Decreases autophagy and apoptosis → necrosis
- Increases ROS generation/ox stress
- Decreases Sirtuin 1 activity
- Lipid accumulation – foam cells
- Major contributing cause of E.R. Stress
Mtb has many pathogenic mechanisms in common with DIABETES

- Like DM, TB perverts **pattern recognition receptor** (PRR)-initiated macrophage signaling, increasing inflammation and tissue damage
  - With DM, **advanced glycolation end-products** (AGEs) are ligands for many PRRs, including the receptors for AGEs (RAGE), TLRs, and NLRs.

- Like DM, Mtb disrupts **macrophage lipid metabolism and increased uptake of ox-LDLs** by scavenger receptors →
  - M2 polarization and
  - Foam cell development

- Both also cause cellular stresses:
  - **Oxidative stress**
  - **Endoplasmic reticulum stress** (ERS) with unfolded protein reaction (UPR) also enhances Mtb survival
  - Leads to release of host cell molecules (DAMPS) also reacting with PRRs in a vicious cycle of more stress, inflammation, and necrosis
Modification of β-Defensin-2 by Dicarbonyls
Methylglyoxal and Glyoxal Inhibits Antibacterial and
Chemotactic Function In Vitro

PLOS ONE | DOI:10.1371/journal.pone.0130533  August 5, 2015

Dicarbonyl modification of
cationic antimicrobial peptides  --
a potential link between hyperglycemia and
increased susceptibility to infection in undiagnosed
and uncontrolled Type 2 diabetes
Endoplasmic Reticulum Stress Controls M2 Macrophage Differentiation and Foam Cell Formation


Background: The interplay of lipid signaling with macrophage phenotype is critical for vascular disease progression.

Results: ER stress links scavenger receptor signaling to macrophage phenotype and foam cell formation through a JNK- and PPAR_γ-dependent pathway.

Conclusion: ER stress is a functional switch controlling macrophage phenotype and cellular cholesterol content.

Significance: Suppression of ER stress is a potential therapeutic target to reduce atherosclerosis progression.
Endoplasmic Reticulum Stress Pathway-Mediated Apoptosis in Macrophages Contributes to the Survival of Mycobacterium tuberculosis

In Raw264.7 murine macrophages, Mtb infection increased levels of ER stress indicators in a time-dependent manner.

Subsequently, UPR is initiated and ROS, NO, & CHOP expression were significantly increased.

Increased Mtb survival is mediated by the eIF2α/CHOP, pathway – and then caspase-12 is induced to cause apoptosis with release of viable mycobacteria.

ER stress pathway plays an important role in the pathogenesis and persistence of mycobacteria.
• Are potential new interventions available to begin testing NOW?
## Molecular Targets of High Interest for TB HDT

### Evidence for PG role or benefit
- AMPK
- P13K-AKT-mTOR
- Sirtuins – STACs
- MAPKs – JNK, ERK
- Rho/ROCK
- Protein kinases: abl, c-kit, VEGF, EGFR, JAK/STAT
- Mevalonate pathway
- Autophagy inducers
- Cathelicidin/AMPs – HDACs
- Wnt/beta-catenin
- MMPs

### NOT YET EXPLORED for PG or benefit
- PARPS
- Angiotensin II receptors
- Hedgehog
- Notch
- HIF-1alpha
- Other kinases – SIK, TAK-1, Src, S6, etc.
- Inflammasomes
- DPP-4
- IDO inhibitors
- GSK-3
- **Many** more/combinations
Approved Targeted HDT Drugs to Evaluate - DM/ TB

• **Signaling/metabolic pathways, for example** - Approved
  – **AMPK+** (*metformin*)
  – Lipid metabolism agents (*statins* and many others)
  – Peptidyl dipeptidase-4 Inhibitors (sitagliptin)
  – Tyrosine kinase inhibitors (*imatinib* and many others)
  – *PARP inhibition* (olaparib)/sirtuin activators (resveratrol/STACs)
  – Rho/ROCK inhibitors (fasudil)
  – Autophagy and ERS (phenylbutyrate with/without vitamin D)
  – Angiotensin II Receptor Blockers (MANY)

• ERS prevention/reduction
• Oxidative stress reduction
• Scavenger receptor suppression
• DAMP antagonists
In *Mtb*-infected mice, adjunctive metformin (MET) reduces the intracellular growth of DS a DR *Mtb* in an AMPK–dependent manner.

MET increased production of mitochondrial reactive oxygen species and facilitates phagosome-lysosome fusion.

MET reduced inflammation and lung pathology, and enhanced specific immune responses and efficacy of conventional TB drugs.

Collectively, these data indicate that MET is a promising candidate host-adjunctive therapy for improving TB treatment.
Simvastatin increases the in vivo activity of the first-line tuberculosis regimen

J Antimicrob Chemother 2014; 69: 2453–2457

Relative to the standard oral regimen of R/H/Z, addition of 25 mg/kg simvastatin in a BALB/c mouse model reduced lung cfu by an additional **1 log$_{10}$ at Day 28** (P=0.01) and by a further **1.25 log$_{10}$ at Day 56** (P=0.01)
Statins

HIGHLY PLEIOTROPHIC EFFECTS

• AGE/RAGE effects
  – Decreases MPO-dependent AGE generation
  – Controls AGE-mediated histone modification
  – Decreases RAGE expression
  – Displaces RAGE from membrane

• Decreases LOX-1 expression and function

• Enhance Efflux pumps for lipids

• Matrix metalloproteinase down-regulation

• Inhibits Kv1.3 channel in T cells - anti-inflammatory

And Others - Will all of these be sufficiently additive to significantly impact TB-DM treatment outcomes?
Gliptins - sitagliptin, a dipeptidyl peptidase-4 inhibitor

DDP-4 has role in inflammation/oxidative stress

• Gliptins decrease “metaflammation”
  – Increasing glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptors
  – AMPK/Sirtuin1 activity in neuro disease models
  – Anti-oxidant molecules

• Suppresses MMP-1, IL-6, inflammasomes/IL-1β, JNK signaling

• Decreases RAGE levels and protein/lipid oxidation

• Maintains CXCL10 expression/T cell trafficking (in tumor microenvironments)
  – Now often given in combination with metformin
• Does a research “gap” exist?

NO

- It is more like the Mariana Trench
Why Needed?

1) The increasing T2DM epidemic in high TB burden regions is a major global TB control problem – increasing TB rates and making therapy less effective

2) Gaps – Limited knowledge of the synergistic PG mechanisms, lack of research to develop optimized/new treatments, and very few clinical trials have addressed improved dual therapy

3) Opportunities - To discover dual PG mechanisms and targets with new research tools and to innovatively adapt existing candidate HDTs to improve outcomes

4) Minimal support available globally for research on pathogenesis and improving therapeutic outcomes
DM-TB NIAID/NIDDK Grants

Total active grants including “DM and TB” = 4
• Observational only = 2 (R01, K23)
• Addressing immunity = 2 (R01, R21)
• Trial Planning = 1 (R34)

Metformin vs. insulin for TB/DM treatment
Ongoing Research for DM/TB – Pathogenesis and Interventions

• **Gates** - ? None

• **EU** – “Tandem” Study Group grant –
  Current funding ends in early 2017

• **Others** – No pharmaceutical interest due to lack of incentive
Future TB/T2DM Research

Opportunity to blunt the effects of the converging epidemics by addressing these obvious but neglected research gaps

We need a roadmap...
Innovate or Fail
DAMPs - Death-Associated Molecular Patterns

Released from damaged/dying cells (innate immunity)

- **S100A Calcium Binding Proteins** (e.g. S100A 8 + 9)
  
  24 total - normally have many critical roles in cellular process, but if released with cell stress/damage – become DAMPs
  
  – Increases neutrophilic infiltration/TB lung damage – one reason why TB lung pathology worse with DM

- **High Mobility Group Box-1 HMGB1**

  Normally nuclear protein, but if released - acts as DAMP
  
  – Enables inflammasome assembly (with NLRs) $\rightarrow$ capase1 $\rightarrow$ IL-1 and IL-18 secretion
  
  – Assists in leukocyte recruitment

- **Both are **ligands for RAGE and TLRs**, increasing signaling and effects** $\rightarrow$ cycle
**Transformative New Technology**

**SINGLE-CELL** high-throughput applications of these assays –

Current –
- DNA seq + RNA seq

Soon to come –
- DNA seq + RNA seq + epigenomic assay(s) – ChIP-based

In development –
- Addition of proteomic assays
- Protein-protein interaction assays

**Results:**
Correlating changes in specific cell’s signaling (and influence of genetics, epigenetics)
→ gene expression (transcription/translation/post-translational modifications)
→ effects on cell metabolism and defense functions (phenotype)
**DAMPs**

**PRR**

**JNK**

**ERK**

**ROS**

**OxLDL**

**SR**

**ER**

**STRESS**

**PARP**

**OxLDL**

**NF-κB**

**AMPK**

**SIRT**

**Foamy Macrophage M2**

**cAMP**

**PKA**

**PI3K**

**AKT**

**PTEN**

**mTOR**

**RTK**

**β-AR**

**Adenylate Cyclase**

**AMP**

**PDE**

**Autophagy**

**Anti-inflammatory**

**PRO-inflammatory And Cell Death**
INTERVENTIONS

Reversing these “metainflammatory” processes

Can they improve treatment for both DM and TB?
Fasudil – rho/ROCK inhibitor

Approved in several nations for treatment of SAH and stroke (vasodilator/anti-inflammatory) but has highly pleiotropic effects – studied for many diseases

• Reduces oxidative stress and ROS production
• Decreased AGE-induced NF-κB-dependent transcriptional activity
• Attenuates high glucose-induced MCP-1 and VCAM-1 expression and monocyte-endothelial cell adhesion
• Enhances autophagosome formation/autophagy
• inhibits ER stress/unfolded protein response

Statins also have some effect on ROCK signaling and seem to work additively with fasudil
Vitamin D (+/- phenylbutyrate*)

• Expression of cathelicidin and β-defensin-2 (antimicrobial peptides)
• Activates LXR-alpha/AMPK pathway
  – Decreases lipogenesis
  – Facilitates lipid egress
  – Decreases NF-kB expression/ROS
• Down-regulates mTOR - Autophagy
• Enables IL-32 activity against Mtb
• Down-regulates MMPs
Sodium Phenylbutyrate

Sodium phenylbutyrate (PB) is FDA-approved for treatment of urea-cycle disorders

- **HDAC inhibitor** - Induction of *cathelicidin* and further induction of autophagy
- Increases **CREB** expression $\rightarrow$ PGC-1α $\rightarrow$ decreases oxidative stress/improves mitochondrial function
- **Reverses endoplasmic reticulum stress** as a chaperone molecule reducing mis-folded protein
  - Decreased nuclear translocation of nuclear factor κB, inflammatory cytokine levels, Toll-like receptor 4 expression, histologic inflammation, and enhanced IL-10 levels.
Phosphodiesterase (PDE) Inhibition

Increased cAMP levels

– Decreases TNF-α expression
– May modify RAGE – by displacing from membrane
– PDE Type 4 inhibition may decrease MMP (1+2) expression, esp. if TNF-induced

Candidate drug - Ibudilast – (esp. for PDE-4)

• Approved in Japan
• in six ongoing Phase II/III trials – MS, migraine, drug dependency (MediciNova)
Angiotensin II Receptor Blockers

Telmisartan or others

Approved drug for hypertension

- Inhibits RAGE/HMGB1 axis expression
- Decreases LOX-1 effects
- Activate SIRT1/AMPK AND PPARy agonist
- decreases - inflammation
  - ROS/oxidative stress
    (reduces TLR-4 up-regulation)
  - lipid accumulation
Nicotinamide (NAM), NR, and NMN
(Nicotinamide riboside or mononucleotide)

Available as dietary supplements for hyperlipidemia

- Tends to decrease inflammation and oxidative stress/ROS
  - Inhibits cAMP phosphodiesterase
  - Inhibits PARPs & pro-inflammatory signaling/Activates sirtuins
  \[ \rightarrow \text{decreases NF-κB target gene transcription} \]

- With or without doxycycline – used to control inflammation/steroid-sparing
  - Tetracyclines (mino) are inhibitors of PARP-1 as well as of MMPs
PARPs – Poly(ADP-Ribose)Polymerases

Critical regulator of inflammation and metabolism
When overactive – e.g., with DM

- **Depletes** NAD+ → *inhibits SIRTs*, mitochondrial dysfunction
- **Increases inflammation** – transcription of key gene targets
  - → NF-kB → CKs, CCs, adhesins...

- **Metabolic abnormalities**, lipid accumulation, ↑ROS

PARP Inhibitors

- Several in clinical trials for cancers - approved - *olaparib*, AND inflammatory diseases,
- Preclinical evidence for reducing **DM** complications and atherosclerosis

**PZA may be a PARP inhibitor!**
**Dimethyfumarate - DMF/MMF**

FDA-approved for MS

- Inhibition of **NF-kB** nuclear translocation
- Activates NRF2/Keap pathway – *antioxidant*
- Induces **heme oxidase-1** →
  - Protects macrophages against mycobacteria
  - Translocation of **HMGB1** from nucleus to cytosol is significantly inhibited by HO-1 inducers (LPS)
  - Upregulates ABCA1 and ABCG1 lipid pumps and SR-B1 to reverse lipid accumulation
  - **CO (carbon monoxide!) Effects** – **Modulates ERS** and decreases inflammation and cell death and increases cellular antioxidants* and induces autophagy
Metformin

• **Decreases glycolation** (pyridoxamine and buformin may be better) reacts with dicarbonyls
• Decreased TNF and other inflammatory CKs by inhibiting ERK/EGR-1 pathway and suppressing scavenger receptors (CD36 and SR-A)
• **AMPK activation**
  – Inhibits mTOR – enhances **autophagy** in TB infected macrophages mediated by PPAR-gamma
  – Inhibits PARP activation and activates Bcl-6
  – Decreases effects of RAGE signaling on some cells and inhibits HMGB1 release
  – Decreases NF-kB expression/Suppresses **ROS** formation
ERS – Being Ignored?

Insulin is a major anabolic hormone and so **insulin resistance may help the ER to cope** by transiently shutting off synthetic pathways. In fact, perhaps one can even consider that **insulin resistance is a bona fide component of the UPR**.

If true, then a critical consideration is that **therapeutic strategies that achieve insulin sensitivity without addressing an underlying ER dysfunction, or JNK activation, or other inflammatory stresses may not be sustainable or even beneficial** in the long term because they would increase the burden on the ER.

Perhaps there should be a **revision of the classic models on which therapeutic strategies for treating obesity, type 2 diabetes, and atherosclerosis are predicated**.
**Highest Incidence of TB Associated with DM**

<table>
<thead>
<tr>
<th>Country</th>
<th>TB incidence (all age groups)/100,000</th>
<th>Adults with DM Million</th>
<th>Population attributable fraction of DM for adult TB cases %</th>
<th>Adult TB cases associated with DM, n</th>
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<td>India</td>
<td>176</td>
<td>65</td>
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</table>

TB = tuberculosis; DM = diabetes mellitus.

DM is Associated with Increased Progression to Active TB and Unfavorable Clinical Outcomes

Hodgson K, et. al. Immunology. 2015 Feb;144(2):171-85