Influence of pre-diabetes on the immunity to pulmonary tuberculosis and latent tuberculosis

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Immunology of TB-diabetes
Studies from the lab

• PTB-DM is associated with an expansion of pathogen specific Th1 and Th17 responses (Kumar et al., JID 2013)
• PTB-DM is associated with enhanced circulating levels of Type 1, Type 17 and other pro-inflammatory cytokines (Kumar et al., AATS 2013)
• PTB-DM is associated with heightened plasma levels of HO-1 and TIMP-4 and elevated absolute neutrophil counts (Andrade et al., Chest 2014)
• PTB-DM is associated with altered CD8+ T and NK cell function including increased frequency of cytokine producing cells (Kumar et al Immunology 2015)
• Coincident DM is associated with decreased frequencies of classical and intermediate monocytes and decreased frequencies of myeloid and plasmacytoid DCs in PTB, LTB and NTB (Kumar et al., Immunology 2015)
Both PTB-DM and LTB-DM are associated with diminished levels of IL-10 family of cytokines (except IL-22) (Kumar et al., Tuberculosis 2015). Coincident DM is associated with diminished systemic and antigen-specific Type 1, Type 17 and other pro-inflammatory cytokines in latent TB (Kumar et al., JID 2014). LTB-DM is associated with decreased frequencies of TB antigen specific Th1, Th2 and Th17 responses (Kumar et al., EJI 2016). LTB-DM is also associated with decreased cytokine production and enhanced cytotoxicity of CD8+ T cells (Kumar et al., JID 2016). PTB-DM or LTB-DM is associated with diminished circulating levels of adiponectin and adipisin and/or enhanced circulating levels of leptin, visfatin and PAI-1 in comparison to PTB or LTB alone (Kumar et al., Cytokine 2016).
Pre-diabetes

• Pre-diabetes (PDM) or intermediate hyperglycemia is a high risk state for diabetes that is defined by glycemic variables that are higher than normal but lower than diabetic thresholds

• The prevalence of PDM is increasing worldwide, and it is estimated that over 470 million people will have PDM by 2030

• PDM is associated with the simultaneous presence of insulin resistance and pancreatic beta-cell dysfunction - abnormalities that start before changes in glycemic control are detectable

• Typically 5–10% of individuals with PDM become diabetic every year, although the conversion rates vary with population characteristics and definition of PDM
Study Objectives

• To identify the influence of coincident pre-diabetes on the systemic and antigen – stimulated cytokine levels in pulmonary tuberculosis

• To identify the influence of coincident pre-diabetes on the systemic and antigen – stimulated cytokine levels in latent tuberculosis
Study Groups

**Pulmonary TB (PTB)** - PTB was diagnosed on the basis of sputum smear and culture positivity

**Latent TB (LTB)** - LTB diagnosis was based on TST > 12mm and Quantiferon TB-Gold ELISA positivity, absence of chest radiograph abnormalities or pulmonary symptoms and negative sputum smears

**Pre-Diabetes (PDM)** – Pre-diabetes was diagnosed on the basis of glycated hemoglobin (HbA1c) levels between 5.7 – 6.4% (American diabetes association)
PROTOCOL

Study group

Pulmonary TB with pre-diabetes (PDM) (n=48)

Pulmonary TB with no diabetes (NDM) (n=42)

Plasma or QFT supernatants

• Type 1 cytokines (IFN$\gamma$, IL-2 and TNF$\alpha$)
• Type 2 cytokines (IL-4 and IL-5)
• Type 17 cytokines (IL-17A, IL-17F and IL-22)
• Regulatory cytokines (IL-10 and TGF$\beta$)
• IL-1 family cytokines (IL-1$\alpha$ and IL-1$\beta$)
• Type 1 Interferons (IFN$\alpha$ and IFN$\beta$)
• Pro-inflammatory cytokines (IL-6, IL-12 and GM-CSF)
PTB-PDM is associated with increased circulating levels of Type 1 and Type 17 cytokines.
PTB-PDM is associated with increased circulating levels of Type 2 and regulatory cytokines.

Cytokine Levels (pg/ml)

<table>
<thead>
<tr>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-10</th>
<th>TFGβ</th>
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<tbody>
<tr>
<td>PDM</td>
<td>NDM</td>
<td>PDM</td>
<td>NDM</td>
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- **IL-4**: $p = 0.0479$
- **IL-5**: $p = 0.0016$
- **IL-10**: $p = 0.0192$
PTB-PDM is associated with increased circulating levels of other pro-inflammatory cytokines

<table>
<thead>
<tr>
<th>Cytokine Levels (pg/ml)</th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IFNα</th>
<th>IFNβ</th>
<th>IL-6</th>
<th>IL-12</th>
<th>GM-CSF</th>
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<td>PDM</td>
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<td><strong>IL-1 Family</strong></td>
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<td><strong>Type 1 Interferons</strong></td>
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<td><strong>Pro-inflammatory Cytokines</strong></td>
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- p = 0.0076
- p < 0.0001
- p = 0.0385
PTB-PDM is associated with increased baseline levels of pro-inflammatory cytokines.

Baseline Cytokine Levels (pg/ml)

- **IFNγ**
  - PDM: 
  - NDM: 
  - **p = 0.0052**

- **TNFα**
  - PDM: 
  - NDM: 
  - **p = 0.0012**

- **IL-17A**
  - PDM: 
  - NDM: 
  - **p < 0.0001**

- **IL-1β**
  - PDM: 
  - NDM: 
  - **p < 0.0001**
PTB-PDM is associated with increased TB antigen stimulated levels of pro-inflammatory cytokines.
PROTOCOL

Study group

Latent TB with pre-diabetes (LPDM) (n=30)

Latent TB with no diabetes (LNDM) (n=30)

Plasma or QFT supernatants

- Type 1 cytokines (IFN$\gamma$, IL-2 and TNF$\alpha$)
- Type 2 cytokines (IL-4, IL-5 and IL-13)
- Type 17 cytokines (IL-17A, IL-17F and IL-22)
- Regulatory cytokines (IL-10 and TGF$\beta$)
- IL-1 family cytokines (IL-1$\alpha$, IL-1$\beta$ and IL-18)
- Pro-inflammatory cytokines (IL-6, IL-12 and GM-CSF)
Latently Infected Individuals With Pre-DM Exhibit Diminished Circulating Levels of Type 1 and Type 17 Cytokines
Latently Infected Individuals With Pre-DM Exhibit Diminished Circulating Levels of Type 2 and Regulatory Cytokines

Cytokine Levels (pg/ml)

IL-4
IL-5
IL-13
IL-10
TGFβ

Latently Infected Individuals With Pre-DM Exhibit Diminished Circulating Levels of Type 2 and Regulatory Cytokines
Latently Infected Individuals With Pre-DM Exhibit Diminished Circulating Levels of IL-1 Family of Cytokines
LTB-PDM is associated with diminished baseline levels of Type 1 and Type 17 Cytokines

- IFNγ
- TNFα
- IL-17A
- IL-1β
- IL-10
LTB-PDM is associated with diminished *M.tb* antigen stimulated levels of Type 1 and Type 17 Cytokines.

Net Cytokine Levels (pg/ml)

- IFNγ: p=0.0099
- TNFα: p<0.0001
- IL-17A: p<0.0001
- IL-1β
- IL-10: p<0.0001
LTB-PDM is associated with no significant alterations upon mitogen stimulated levels of Type 1 and Type 17 Cytokines.
TB-PDM is characterized by elevated circulating levels of Type 1, Type 17 and other pro-inflammatory cytokines.

TB-PDM is also characterized by increased systemic levels of Type 2 (IL-5) and regulatory cytokines.

Moreover, TB antigen stimulated whole blood also showed increased levels of pro-inflammatory cytokines as well.

Latent tuberculosis with pre-diabetes is characterized by diminished circulating levels of type 1 and type 17 cytokines.

Latent tuberculosis with pre-diabetes was also associated with decreased systemic levels of other proinflammatory cytokines and an anti-inflammatory cytokine (IL-10).

LTB with PDM is also associated with decreased TB antigen stimulated cytokine levels.
Knowledge gaps

- Role of T cells in TB-PDM co-morbidity
- Role of antigen-presenting cells in TB-PDM co-morbidity
- Role of hyperglycemia in the immune response in these individuals
- Role of other metabolic abnormalities in the immune response
- Pressing Question: Why is the immune response diametrically different in PTB and LTB individuals? Is this a reflection of TB disease or is there another underlying factor that is different between the two groups?
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