Glutathione as an immunoadjuvant

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Associate Professor
TB meningitis
GLUTATHIONE

A Glutamyl-cysteine ligase (GCLM/GCLC)

\[
\text{Glutamate} + \text{Cysteine} \rightarrow \gamma\text{-glutamyl cysteine} + \text{H}_2\text{O}
\]

B Glutathione Synthase (GSS)

\[
\gamma\text{-glutamyl cysteine} + \text{Glycine} \rightarrow \text{Glutathione} + \text{H}_2\text{O}
\]

C Glutathione Reductase (GSR)

\[
\text{Glutathione} \rightarrow \text{Oxidized glutathione (GSGG)} \quad \text{Reduced form of GSH (rGSH)}
\]

NAC BSO
Figure IA and Table I: Growth of *M. tuberculosis* in the presence and absence of GSH as determined by measuring OD and CFU.

**Table 1:** Growth of H37Rv in 7 Hg medium

<table>
<thead>
<tr>
<th>H37Rv treatment</th>
<th>Mean no. of CFU ± SD on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>None</td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>± $8.81 \times 10^3$</td>
</tr>
<tr>
<td>5 mM GSH</td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>± $1.2 \times 10^3$</td>
</tr>
</tbody>
</table>

Venketaraman *et al*; Infect Immun 2005

Lagman *et al*; PLoS One 2015
Survival of H37Rv inside monocytes co-cultured with NK cells.

Figure 5A

Millman et al; J Interferon Cytokine Res 2008

Allen et al; Front Immunol. 2015
Survival of H37Rv inside monocytes co-cultured with T cells.

Guerra et al; PLoS One 2011
Assay of GSH in macrophages, T cells and NK cells lysates from healthy and HIV-infected subjects

Guerra et al; Clin Dev Immunol 2012

Guerra et al; PLoS One 2011

Guerra et al; Clin Exp Immunol 2012

Guerra et al; Clin Dev Immunol 2012
Quantifying GCLC (Panel 1) and GSS (Panel 2) enzyme levels in Healthy and HIV-infected subjects.

**Panel 1**

**A**

Relative intensity/ (GAPDH)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Healthy</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>91kDa</td>
<td><img src="image1.png" alt="Image" /></td>
<td>*</td>
</tr>
<tr>
<td>73kDa</td>
<td><img src="image2.png" alt="Image" /></td>
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</tr>
<tr>
<td>48kDa</td>
<td><img src="image3.png" alt="Image" /></td>
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</tr>
<tr>
<td>38kDa</td>
<td><img src="image4.png" alt="Image" /></td>
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</tr>
<tr>
<td>34kDa</td>
<td><img src="image5.png" alt="Image" /></td>
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</tr>
</tbody>
</table>

**Panel 2**

**A**

Relative intensity/ (GAPDH)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Healthy</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>91kDa</td>
<td><img src="image6.png" alt="Image" /></td>
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</tr>
<tr>
<td>52kDa</td>
<td><img src="image7.png" alt="Image" /></td>
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<tr>
<td>48kDa</td>
<td><img src="image8.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>38kDa</td>
<td><img src="image9.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>34kDa</td>
<td><img src="image10.png" alt="Image" /></td>
<td></td>
</tr>
</tbody>
</table>

*Morris et al; Frontiers Pharmacol 2014*
Quantifying the levels of GSR (Panel A) and *Mtb* survival (Panel B) in HIV-positive subjects.

Morris *et al*; *Frontiers Pharmacol* 2014

Morris *et al*; *J Interferon Cytokine Res* 2013
Assay of IL-12, IL-2, IFN-γ, IL-10 and TGF-β in plasma samples from healthy and HIV positive individuals.

Ly et al; J Interferon Cytokine Res 2015
Baseline comparison of the interleukin-6 (IL-6) and reactive oxygen species (ROS) markers between healthy volunteers and HIV-positive individuals.

**Plasma**

- **A**
  - **Healthy Baseline**
  - **HIV Baseline**

- **B**
  - **Healthy Baseline**
  - **HIV Baseline**

**RBCs**

- **C**
  - **Healthy Baseline**
  - **HIV Baseline**

**Monocytes**

- **D**
  - **Healthy Baseline**
  - **HIV Baseline**

*Ly et al; J Interferon Cytokine Res 2015*
Changes in the levels of plasma cytokines (IL-12, IFN-γ, IL-10, IL-6 and TGF-b), free radicals, GSH, and *Mtb* survival in HIV positive subjects at pre- and post-GSH supplementation.
Cycle of HIV induced oxidative stress

- ↓ GSH
- ↓ IL-6, IL-2, IFNγ
  - Directs to T_{eff} response
- ↓ IL-17, TNF-α
  - Inhibition of granuloma formation
- ↓ IL-1β
  - Lowered antimicrobial activity in macrophages
- ↑ IL-10
  - Macrophage deactivation
- ↑ TGFβ
  - Limits T-cell proliferation

**HIV**

IL-6 overproduction

**Oxidative stress**

GSH supplementation

**Control of M. tb infection.**

**Control of AIDS progression.**

Ly et al; J Interferon Cytokine Res 2015
Second clinical trial in HIV positive subjects with low CD4 T cell counts

Valdivia et al; AIDS Res Hum Retroviruses. 2017
Second clinical trial in HIV positive subjects with low CD4 T cell counts

Valdivia et al; AIDS Res Hum Retroviruses. 2017
Summary of our clinical trial findings:

Active immune responses and control of *M. tb.* infection inside the granuloma

Active *M.tb.* Infection inside the granuloma
Assay of total GSH and rGSH in brain lysates from healthy, AD, and HIV-1 positive subjects.

Saing et al, BBA Clin 2016
Assay of MDA in brain tissues from healthy, AD, and HIV-1-positive subjects.

Saing et al, BBA Clin 2016
Western blot analysis of a GSH de novo synthesis enzyme, GCLC, in brain tissue from healthy subjects

A

Glutamate-cysteine ligase catalytic subunit (GCLC)

B

Saing et al, BBA Clin 2016
Western blot analysis of GSS, a GSH *de novo* synthesis enzyme in brain tissues derived from healthy, AD and HIV-1-positive subjects

Saing *et al*, BBA Clin 2016
Western blot analysis of a recycling enzyme, GSR, in brain lysates from healthy, AD and HIV-1-positive subjects

Saing et al, BBA Clin 2016
Other related studies


Clinical trial in TB patients. Aurum Institute
Effects of NAC in granulomatous responses against *Mtb* infection
Synergistic effects of antibiotics+GSH in improving granulomatous responses against *Mtb* infection
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- Judy Ly
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- Leslie Gonzalez

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