Mycobacterium leprae infection triggers a type I interferon-dependent oligoadenylate synthetase-like (OASL) anti-microbial gene
Approaches used in our research group: functional genomics and genetic epidemiology (fungen-genepi)

- **Functional genomics**
  - microarrays, reanalysis of GEO data and gene expression in low/moderate scale

- **Genetic epidemiology**
  - case-control studies, meta-analysis
  - validation of candidate genes

- **Confirmation**
  - of the association and pathways of susceptibility and resistance
  - functional validation

- **Confirmation of pathways of susceptibility and resistance**

**Hidden challenges**
Type I interferon signature in *M. leprae* infection

*M. leprae* (live) → Mycobacterial infection → Primary Schwann cells → Global gene expression → Type I Interferon signature → **OASL**: the most expressed gene during *M. leprae* infection.
Type I interferon signature in mycobacterial diseases

An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis

Matthew P. R. Berry1, Christine M. Graham1, Finlay W. McNab1, Zhaohai Xu2, Susannah A. A. Bloch1, Tolu Oni1,2, Katalin A. Wilkinson1,2, Romain Banchereau2, Jason Skinner2, Robert J. Wilkinson1,2, Charles Quinn4, Derek Blakenship5, Ranju Dhawan6, John J. Cush8, Asuncion Mejas10, Octavio Ramilo10, Onn M. Kon3, Virginia Pascual10, Jacques Banchereau2, Damien Chausabel10 & Anne O’Garra1

Genome-Wide Expression Profiling Identifies Type 1 Interferon Response Pathways in Active Tuberculosis

Tom H. M. Ottenhoff2, Ranjeeta Hari Dass3, Ninghan Yang4, Mingzi M. Zhang1, Hazel E. E. Wong1, Edhyana Sahiratmadja4, Chiea Chuen Khor1, Bachti Aitjahbana1, Reinout van Crevel1, Sangkot Marzuki5, Mark Seidelstad5, Esther van de Vosse5, Martin L. Hibberd1

Type I Interferon Suppresses Type II Interferon–Triggered Human Anti-Mycobacterial Responses

Rosane M. B. Teles,1 Thomas G. Graeber,1 Stephan R. Krutzik,1 Dennis Montoya,1 Mirjam Schenk,1 Dolphine J. Loo,1 Evangelia Komissopoulou,1 Kindra Kelly-Scumpia,1 Rene Chun,3 Shankar S. Iyer,2 Eузenir N. Samo,6 Thomas H. Rea,7 Martin Hewison,7 John S. Adams,7 Stephen J. Popper,7 David A. Reisman,8 Steffen Stingger,10 Barry R. Bloom,11 Gehong Chong,11 Robert L. Modlin1,2
Type I IFN signature gene expression in mdTHP-1 cells infected with viable *M. leprae*.

Normalized expression values (deltaCt) of described genes in mdTHP-1 cells infected with viable *M. leprae* at MOIs of 10:1 and 100:1 for 3h, 24h and 48h. Results are represented as mean ± SEM of 3 or more independent biological replicates.
OASL mRNA and protein expression in mdTHP-1

Normalized OASL mRNA expression levels (deltaCt) in THP-1 cells infected with either *M. bovis* BCG, irradiated or viable *M. leprae* at MOIs of 10:1 and 100:1 for 48 hours. Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by ANOVA followed by Bonferroni’s multiple comparison test (*** p<0.0001; * p<0.05). Western Blots verifying protein levels in the same mdTHP-1 cultures used for mRNA expression.
Immunocytochemistry detecting Alexa 633 labeled OASL protein in THP-1 cells infected with either viable *M. leprae*, PKH green labeled irradiated *M. leprae* or GFP labeled BCG (d). Nuclei are labeled by DAPI. Scale bar: 10 µm. Quantitative analysis of the immunocytochemistry. Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by ANOVA followed by Bonferroni’s multiple comparison test (*** p<0.0001; ** p<0.001).
**M. leprae** DNA triggers cytoplasmic DNA sensing pathway

Normalized OASL and IFIT1 mRNA expression (deltaCt) of mdTHP-1 cells at 24h post-transfection with lipofectamine alone (control), *M. leprae* DNA or RNA (1μg/4x10⁵ cells) and *M. leprae* DNA treated with DNase. Cells were also treated with nucleic acid without transfection reagent. Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by ANOVA followed by Bonferroni’s multiple comparison test (** p<0.001). Confocal microscopy image showing *M. leprae* cell wall components in mdTHP-1 phagosomes (RAB7 and LAM positive labeling; arrow) and cytoplasm (RAB7 negative labeling and LAM positive labeling; arrowhead). Normalized mycobacterial ESAT-6 mRNA expression levels in mdTHP-1 cells infected with viable *M. leprae* at MOIs of 10:1 and 100:1.
Many pathways to trigger type I interferon . . .
M. leprae activates the type I interferon pathway in a STING/TBK1/IRF3 manner

Normalized OASL expression (deltaCt) of mdTHP-1 cells stimulated with CpG (TLR9 agonist; 1 µM) or infected with viable M. leprae alone or in the presence of E6446 (TLR9 antagonist; 2 µM). (e) Normalized OASL expression (deltaCt) of mdTHP-1 cells lipofected with c-di-AMP (STING ligand; 1 µg/ml) or infected with viable M. leprae alone or in the presence of BX795 (TBK1 inhibitor; 6 µM). Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by two-tailed Student’s t test (* p=0.01; ** p=0.001; *** p=0.0006).
**M. leprae** activates the type I interferon pathway in a STING/TBK1/IRF3 manner

Imunofluorescence for nuclear IRF3 nuclear detection in mdTHP-1 cells infected with live *M. leprae* at MOI 100:1. Quantitative analysis of the imunofluorescence. Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by two-tailed Student’s *t* test.
OASL gene silencing affects the release of an important chemokine and hinders *M. leprae* viability

Normalized gene expression measurements for OASL in THP-1 cells transfected with siRNA for OASL and infected with viable *M. leprae* for 24 or 48 hours. *M. leprae* viability as given by the ratio of 16S RNA/DNA in control and OASL siRNA transfected THP-1 cells following 24 or 48 hours of infection; Detection of CCL2/MCP-1 in the supernatants of silenced THP-1 cultures. Results are represented as mean ± SEM of 3 or more independent biological replicates and statistical significance was calculated by ANOVA followed by Bonferroni’s multiple comparison test (* p<0.05).
Induction of type I interferon is crucial to the success of mycobacterial infection

THP-1 cultures infected with BCG or BCG+DNA for 72h and (a) viability was measured by the ratio of 16S RNA/DNA in control; (b) Detection of CCL2/MCP-1 in the supernatants of THP-1 cultures infected with BCG or BCG+DNA. Results are represented as mean ± SEM of 3 independent biological replicates and statistical significance was calculated by two-tailed Student’s t test (* p=0.05).
Genetic analysis confirms functional association

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype/Allele</th>
<th>N (frequency %)</th>
<th>Logistic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>rs2258227</td>
<td>AA</td>
<td>318 (0.61)</td>
<td>311 (0.62)</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>168 (0.32)</td>
<td>171 (0.34)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>35 (0.07)</td>
<td>16 (0.03)</td>
</tr>
<tr>
<td></td>
<td>Allele A</td>
<td>804 (0.77)</td>
<td>793 (0.80)</td>
</tr>
<tr>
<td></td>
<td>Allele T</td>
<td>238 (0.23)</td>
<td>203 (0.20)</td>
</tr>
<tr>
<td></td>
<td>T Carriers</td>
<td>203 (0.39)</td>
<td>187 (0.37)</td>
</tr>
<tr>
<td>rs4403877</td>
<td>GG</td>
<td>322 (0.74)</td>
<td>360 (0.76)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>104 (0.24)</td>
<td>107 (0.22)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>8 (0.02)</td>
<td>9 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Allele G</td>
<td>748 (0.86)</td>
<td>827 (0.87)</td>
</tr>
<tr>
<td></td>
<td>Allele A</td>
<td>120 (0.14)</td>
<td>125 (0.13)</td>
</tr>
<tr>
<td></td>
<td>A Carriers</td>
<td>112 (0.26)</td>
<td>116 (0.24)</td>
</tr>
<tr>
<td>rs3213545</td>
<td>GG</td>
<td>327 (0.62)</td>
<td>266 (0.53)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>176 (0.34)</td>
<td>207 (0.41)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>21 (0.04)</td>
<td>33 (0.07)</td>
</tr>
<tr>
<td></td>
<td>Allele G</td>
<td>830 (0.79)</td>
<td>739 (0.73)</td>
</tr>
<tr>
<td></td>
<td>Allele A</td>
<td>218 (0.21)</td>
<td>273 (0.27)</td>
</tr>
<tr>
<td></td>
<td>A Carriers</td>
<td>197 (0.37)</td>
<td>240 (0.47)</td>
</tr>
</tbody>
</table>
Investigation of the role of *OASL* in leprosy: case-control association study

Normalized gene expression values for the *OASL* gene in nerve biopsies obtained from leprosy suspects diagnosed as leprosy (L; n=25) or other non-leprous neuropathy (NL; n=26), and stratified by SNP rs3213545 genotypes as GG homozygotes or A allele carriers. Results are represented as mean ± SEM and statistical significance was calculated by non-parametric Mann-Whitney-Wilcoxon test (* p=0.026).
In conclusion . . .

- This work provides the first evidence that OASL mRNA expression is up-regulated early on the infection of the host cell by *M. leprae*.

- *M. leprae*-triggered type I interferon response is an effective strategy of virulent mycobacteria to escape the bactericidal responses.

- OASL should be considered a pivotal gene to both leprosy and other immune-based diseases and may aid in the development of strategies to treat and prevent these diseases.
Acknowledgments

Leprosy laboratory
Euzenir N Sarno
ASA
Ana M Sales
José A Nery
Nádia Duppre
Ximena Ilharramendi

Patologia
Sérgio Antunes

LMC
Cristina Pessolani
Flávio Lara
Luciana Rodrigues

PROCC
Antônio Pacheco

ILSL, Bauru
Ida Batista
Patrícia Rosa

Hansen Program, Baton Rouge
Diana Williams