Reprogramming Adult Schwann Cells to Stem Cell-like Cells by Leprosy Bacilli Promotes Dissemination of Infection

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Background:

- Pathogen and Disease
- Schwann cells

Major findings and figure review:

Summary & question discussion:
Pathogen: *Mycobacterium leprae*

- In 1873, Armauer Hansen discovered the leprosy bacillus in skin biopsies but failed to culture *M. leprae* in the synthetic media.

- Genome sequenced a century later (2000), the nine-banded armadillo was used as a surrogate host.

- Exceptionally slow to growth with a doubling time of ~14 days.

- Reductive evolution resulted in extensive genome decay for *M. leprae* (mostly composed of pseudogenes).

- The complete genome sequence of *M. leprae* contains 3,27Mb.

* M. tuberculosis genome: 4,4 Mb

* M. paratuberculosis* (causative agent of Johne’s disease in ruminants): 4.8 Mb

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Cultivation of *M. leprae*
**M. leprae** infection and prevalence of leprosy

- Leprosy remains an important global health problem and is an infectious neurodegenerative diseases of the peripheral nervous system (PNS) leading to non-traumatic neuropathies in the world.

- Best understood as two conjoined diseases. The first is a chronic mycobacterial infection and the second is a peripheral neuropathy.

Table 2: Trends in the detection of new cases of leprosy, by WHO Region, 2004–2011

<table>
<thead>
<tr>
<th>WHO Region* – Région de l’OMS*</th>
<th>No. of new cases detected – Nombre de nouveaux cas dépistés</th>
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<tbody>
<tr>
<td></td>
<td>2004</td>
</tr>
<tr>
<td>African – Afrique</td>
<td>46 918</td>
</tr>
<tr>
<td>Americas – Amériques</td>
<td>52 662</td>
</tr>
<tr>
<td>South-East Asia – Asie du Sud-Est</td>
<td>298 603</td>
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<tr>
<td>Eastern Mediterranean – Méditerranée orientale</td>
<td>3 392</td>
</tr>
<tr>
<td>Western Pacific – Pacifique occidental</td>
<td>6 216</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>407 791</strong></td>
</tr>
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</table>

* Reports from the European Region are not included. – Les rapports émanant de la Région européenne ne sont pas inclus.
Mechanism of transmission could be via close contact and droplet infection

No effective vaccine

Multi drug treatment is effective to clear leprosy

Symptoms:

Lack of myelin production by infected Schwann cells leads to

- Nerve damage,
- Sensory loss and
- Disfiguration of skin, sadly results in social exclusion.

M. leprae causes leprosy

http://www.leprosy.org
Schwann cells are the principle glial cells of the peripheral nervous system and are the major target for *M. leprae*.

Schwann cells exist in two main forms: a nonmyelinating form, and a myelinating form,
Infection of peripheral nerve by *M. leprae* via blood vessels
Tyrosine kinases: maiming myelin in leprosy

Mitogen/neuregulins

Nerve regeneration, degeneration, cell proliferation etc.

Demyelination occurs through the ErbB2 receptor.

Intracellular life of *M. leprae* in Schwann cells

- Non-myelinated Schwann cell
- Proliferation
- Re-infection
- Demyelination
  - Nerve injury response
  - De-differentiation
  - Proliferation
- Myelin-free de-differentiated Schwann cell pool
- Re-differentiation
- Remyelination
- Propagation of Bacterial niche
- ErbB2 activation
  - Canonical Ras, Raf, Mek, Erk1/2 Signaling
Unresolved questions related to leprosy

1. What are the events beyond Erk1/2 phosphorylation that lead to disassembly of the myelin sheath during *M. leprae* infection (definitely not dependent on the Schwann cell death)?

2. How *M. leprae* spreads from Schwann cells to other cell types, including skeletal and smooth muscle cells during late stages of this chronic disease
Major findings

Masaki et al. identify an unexpected mechanism by which the pathogen triggers its dissemination to various tissue types.

- This study provides evidence that show how *M. leprae* is capable of exploiting the genomic plasticity of the Schwann cell by deleting its identity and reprogramming it other cell types.

- Additionally, they highlighted possible mechanisms for *M. leprae* dissemination through granuloma formation during the course of the disease.
Isolation and purification of Schwann cells

FACS and RT-PCR analysis showed that they are mature dedifferentiated Schwann cells.
Dedifferentiated adult Schwann cells are highly susceptible to *M. leprae*

Transcriptome study showed upregulation of genes related to DNA replication and Epithelial-Mesenchymal Transition (EMT) genes.

By 27 days, infected cells gradually “turn on” numerous developmental genes, comprising mostly the mesoderm development.
Intracellular *M. leprae* removes Sox10 from Schwann cell nucleus. In infected cells, gradual "shut down" of Schwann cell myelination and lineage-associated genes occurs within 4 weeks.
Intracellular *M. leprae* converts Sox2⁺/p75⁺/Sox10⁺ Schwann cell phenotype to Sox2⁺/p75⁻/Sox10⁻ reprogrammed cells.
Reprogrammed Schwann cell exhibits properties similar to progenitor stem cell-like cells (pSLC)

Analyses of p75− cells by microarrays, RT-PCR, qPCR, immunofluorescence (IF), and FACS revealed acquisition of various mesodermal and neural crest markers.

Reprogramming causes significant epigenetic changes in the pSLC.
Fig. 3  pSLC differentiation reprogrammed towards mesenchymal tissues

56% of the transcripts accounted for mesoderm-related targets

Bone differentiation
Adipocyte cells
smooth muscle-like cells

Myotube formation in the presence of C2C12 myoblasts
pSLC indirectly transfer infection to myotubes following differentiation

pSLC spontaneously differentiate into SMA\(^+\) smooth muscle-like cells
Redifferentiation of Reprogrammed pSLC Contributes to Passive Bacterial Transfer to Skeletal Muscles and Smooth Muscles In Vivo

10 days post injection

21 days post injection

Redifferentiation of Reprogrammed pSLC Contributes to Passive Bacterial Transfer to Skeletal Muscles and Smooth Muscles In Vivo
Fig. 5 Reprogrammed pSLC Secrete Immune/Growth Factors that Promote Macrophage Survival and Migration

pSLC produced a range of chemokines, cytokines that are known to be produced by mesenchymal stem cells.

pSLC-CM also influenced increased survival of macrophages with minimum apoptosis (Fig. 5C)
pSLC-CM attracted a significant number of macrophages

Migration capacity was increased in the presence of live pSLC, implying that freshly secreted pSLC factors attracted more MQ

A high dose of CCI to inhibit significant MQ migration indicates a collective effect of immune factors, but not CC-chemokine alone, is required to exert effective macrophage chemotactic ability.
Reprogrammed pSLC Promote Bacterial Dissemination via Macrophages and Granuloma Formation In Vivo

By 3 weeks post-injection, pSLC containing intracellular *M. leprae* spread along the interstitial connective tissues in the perimysium and to the skeletal muscle-dermal interphase (SkMDIP).

Intracellular *M. leprae* were efficiently transferred from donor GFP$^+$ pSLC to GFP$^-$ recipient tissue cells in vivo.

pSLC Contribute to Granuloma-like Formation following bacterial dissemination.

These data suggest both transfer of *M. leprae* from pSLC to macrophages and migration of these infected macrophages from the granuloma site.
These in vitro model of granuloma suggest that chemoattractants released from pSLC could have provided the proper signals for macrophage migration and GLS formation.
Model of *M. leprae* mediated reprogramming Schwann cells to pSLC
Major findings

• During *M. leprae* mediated reprogramming process in the Schwann cells, the transcription factor Sox10 is exported from the nucleus to the cytoplasm, followed by epigenetic changes and downregulation of gene expression.

• Degradation of Sox10 results in dedifferentiation and identity loss of Schwann cells.

• Following identity loss, Schwann cells are reprogrammed to stem-cell-like cells with the ability to redifferentiate into infected mesodermal cells i.e. skeletal and smooth muscles.

• These *M. leprae* infected stem-cell-like cells secrete various immunomodulatory molecules which triggers macrophages to form granulomas-like structures.

• Following granuloma formation, infected macrophages serve as another form of systemic pathogen dissemination.
Discussion questions:

• What does this reprogramming strategy suggest about *M. leprae* and its coevolution with the host?

• Given the fact that *M. leprae* hijacks identity of Schwann cells in vitro, do you think the same would happen in vivo?

• Why only lepromatous form of infection associated with the removal of Sox10? What could be the mechanism for Sox10 export from the nucleus to cytoplasm?
Thank you!