Armadillo: An emerging animal model for leprosy

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EXPANDED RANGE OF ARMADILLO IN USA

150 yrs
Nine banded armadillos (*Dasypus novemcinctus*)

The only natural hosts (other than Human) of *M. leprae*.

- Develops fully disseminated Disease with extensive nerve involvement.
HUMAN INTERECTION

The End
ARMADILLOS: SOURCE OF ZOONOTIC TRANSMISSION OF LEPROSY TO HUMANS

88% of Armadillos and 64% of US Human cases
With endemic exposure Share the Sylvan strain

April 27, 2011  The New York Times
LEPROSY AND ARMADILLO

- **M. leprae**: The causative agent of leprosy
  - Only known bacteria capable to invade nerves and induce neuro-degeneration.
  - Still not cultivable *in-vitro*.
  - Available anti leprosy treatment (MDT) is very efficient bactericidal, DOESN’T HELP IN NERVE DAMAGE.
  - Human nerve biopsy: un-ethical, not suitable for Molecular studies.
  - No other animal model develops leprosy and nerve damage.

- **ARMADILLO**: The only host other than human.
  - The only immunologically intact host of *M. leprae* also recapitulates leprosy as seen in human.
  - Host of choice to propagate *M. leprae* for research purpose.
  - The only animal model to study nerve damage in leprosy.
  - Genetically Identical quadruplets
  - Genome sequence available
CHARACTERIZING THE NEUROPATHY IN ARMADILLOS

- Clinical symptoms
- Nerve Physiology: MNCV and CMAP
- Disseminated Infection
- Morphometry
- Nerve Fiber density
- Comparative Histopathology
- Gene expression profile
  - Global: Cross Species Microarray
  - Selected Marker: Inflammation, Nerve damage and Growth Factors
CLINICAL LEPROSY IN HUMAN AND ARMADILLOS
Similar Ulceration in Human and Armadillo feet

Armadillo feet in late stage of laboratory infection

Human patient feet
NERVE FUNCTION ABNORMALITY

Motor Nerve Conduction Velocity (MNCV) and Compound Motor Action Potential (CMAP)

Standard technique identifies MNCV of <49m/sec as abnormal. Results of our sampling similarly suggest armadillo MNCV <40 m/s and CMAP <0.9mV are out of normal ranges.
Orientation of Posterior Tibial Nerve

- 4-6 cm proximal half of post tibial nerve
- 1-3 cm distal half of post tibial nerve
Degree of Inflammation: leukocyte counts in the posterior tibial nerve
Bacilli count in armadillo nerves by Q-PCR

1.00E+06

1.00E+05

1.00E+04

1.00E+03

1.00E+02

1.00E+01

1.00E+00

Treated with Rifampicin after developing the disease

Not treated after developing the disease

16S rRNA qRT-PCR

RLEP qPCR
De-myelination in infected Armadillo nerves

**Elementary Morphometry**

Total number of myelinated fibers: Infected < naïve

Distribution of fibers: Infected: bimodal, Naïve: Single peak
Epidermal Nerve Fiber Density (ENFD)

Right ear  
Right upper abdomen  
Right leg

immunostained with PGP9.5, a neuronal marker

Lower* ENDF among infected animals

*not statistically significant
GENE EXPRESSION PROFILING OF INFECTED ARMADILLO NERVE

Cross species microarray hybridization

Naïve

Infected

Human microarray (44K)
Nerve damage in leprosy is a complex process involving various pathways
Ingenuity Pathway Analysis

Polyamine Regulation in Colon Cancer
Protein Ubiquitination Pathway
Chemokine Signaling
Virus Entry via Endocytic Pathways
14-3-3-mediated Signaling
B Cell Receptor Signaling
Neuregulin Signaling
Glucocorticoid Receptor Signaling
Mitochondrial Dysfunction
ILK Signaling
Hepatic Fibrosis / Hepatic Stellate Cell Activation
Glioma Signaling
HER-2 Signaling in Breast Cancer
ERK/MAPK Signaling

Chemokine Signaling
Neuregulin Signaling
MAPK Signaling
### Neuronal markers selected for quantitative RT-PCR:

<table>
<thead>
<tr>
<th>Genomic Target</th>
<th>Function</th>
<th>Differential expressed in Condition</th>
<th>Reference:</th>
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<tbody>
<tr>
<td>(GFAP) Glial Fibrillary Acidic Protein</td>
<td>Cell structure, movement, communication, mitosis, and blood brain barrier.</td>
<td>↑ Burn injury</td>
<td>BMC Infect Dis. 2008 Jun 24;8(1):84</td>
</tr>
<tr>
<td>MAG (Myelin-Associated Glycoprotein)</td>
<td>Myelination during nerve regeneration.</td>
<td>↑ Apotransferrin (aTf) Stimulation</td>
<td>J Neurosci:2006; 26:7532-40</td>
</tr>
<tr>
<td>MBP (Myelin Basic Protein)</td>
<td>Myelination of nerves</td>
<td>↑ CNS myelinogenesis</td>
<td>Physiol Behav. 2007 Sep 10;92(1-2):46-53</td>
</tr>
<tr>
<td>N-Cadherin</td>
<td>At certain CNS synapses, presynaptic to postsynaptic adhesion</td>
<td>↑ Regenerating visual structures</td>
<td>Exp. Neurol.2002:177: Page 396-406</td>
</tr>
<tr>
<td>PGP9.5 (UCHL1)</td>
<td>Normal synaptic and cognitive function</td>
<td>↑ Human prostate cancer cells express</td>
<td>Prostate. 2007 Dec 1;67(16):1761-9</td>
</tr>
<tr>
<td>NGF β</td>
<td>Survival and maintenance of sympathetic and sensory neurons.</td>
<td>↑ Traumatic Brain Injury</td>
<td>J Trauma. 2008 Jun 19</td>
</tr>
<tr>
<td>DLK-1</td>
<td>Required for activation of MAPK</td>
<td>↑ Nerve Re generation</td>
<td>Cell 2009;138:1005-1018.</td>
</tr>
</tbody>
</table>
Development of quantitative RT-PCR

✓ Human gene sequence was used as template to Retrieved Armadillo gene sequence
✓ was used to design the Taq Man probe and primers.
✓ Relative quantification by using ΔΔCt method
✓ GAP3DH was used as normalizer
Infected vs Naive

Differential expression of selected nerve marker during the active infection with *M. leprae* compared to naïve Armadillo nerve (n=5)

- Inflammation,
- Upregulation of neuronal component (Active damage / Repair effort)
- No activation of MAPK (no Regeneration)
After treatment

armadillos treated with Rifampicin for one year compared to naïve armadillo

- Consistent Inflammation even after treatment
- No activation of MAPK (no Regeneration)
Intra-neural Inoculation of *M. leprae* in Nine-banded Armadillos (*Dasypus novemcinctus*).
Surgical removal, Orientation and gene expression at various time points after infection

- In deep anaesthesia, post tibial nerve was exposed
- Total $10^7$ *M. leprae* were injected directly into the nerves of 14 armadillos.
- Nerves were surgically removed in pairs at 3, 7, 14, 21, 30, 60 and 90 days post inoculation.

- AFB and histopathology
- Molecular enumeration
- Q-RT-PCR
Greater involvement of distal nerve

- No Acid Fast Bacilli (AFB) in microscopy.
- Total number of bacteria retain in the nerve (~ 9.18+3 \textit{M. leprae}) was significantly lower than natural or intra-venous Infection (9.3E+04 bacilli/mm of nerve).
Higher cytokine production

Down regulation of Regeneration and Growth factors
CONCLUSIONS:

- Nerve damage in leprosy is the result of:
  - Inflammation,
  - Demyelination / degeneration
  - Failure of regeneration efforts

- Armadillo is an animal model for:
  - Study molecular events associated with neuropathy.
  - Reproducing the observation seen in-vitro and other in-vivo intervention studies.
  - Test any neuro-regenerative or protective Agents.
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