Epidermal Nerve Fiber and Schwann cell densities in the distal leg of Nine-banded Armadillos with Experimental Leprosy neuropathy

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Skin innervation

- Unmyelinated (C) and Myelinated (A-fibers) fibers and specialized mechano receptors
- C-fibers end as free nerve endings
- Epidermis: **Small caliber unmyelinated C fibers** — light touch, pain and temperature sensations
- Undetectable by electrophysiological tests
- Invasive nature of nerve biopsies
- Difficulty to discern differences between autonomic & somatic unmyelinated fibers
- 3mm skin punches— to diagnose distal sensory neuropathies
- Autonomic function using sweat gland innervation

**References**

Different components of skin structures can be evaluated.

Axons

Remak Schwann cells

PGP9.5: axons
P75: Schwann cells

All photographs taken at cutaneous nerve laboratory, JHU.
ARMADILLOS

- Nine-banded armadillos are naturally susceptible to *M. leprae*
- Experimental neuropathy: Develop extensive involvement of their peripheral nerves when infected with *M. leprae*
- Recapitulates leprosy neuritis in humans

*M. leprae* in Armadillos PN
- Progressive nerve conduction deficit in compound motor action potential (CMAP; mV; y-axis)
- Complete conduction block in late infection.
- The upper lines: stimulation at the ankle
- Lower ones to the knee.
Nearly all of the animals that developed a conduction deficit exhibited signs of clinical neuropathy in their foot pads. The earliest clinical sign is loss of sensation.
Collaboration between JHU and HRSA/National Hansen's disease programme, USA

Neurophysiology
* National Hansen's disease programme, Baton Rouge

Skin biopsy
*Cutaneous nerve lab, Neurology
Objective

• Assess nerve conduction velocity in *M. leprae* infected animals vs. control animals

• Investigate epidermal nerve fiber density and Schwann cell density in *M. leprae* infected vs. control animals
Methodology

- 11 naïve and 9 *M. leprae*-infected armadillos
- Caldwell Sierra II portable electrophysiology unit
- Surface stimulation of the posterior tibial nerve: Distally at the ankle
  Proximally at the knee
- Motor nerve conduction velocity (MNCV)
- Compound muscle action potential recorded (CMAP)
Methodology-Skin punches

- 3mm skin punches
- Naïve: ears, upper and lower abdomen, distal leg
- M.leprae infected animals: distal leg
- 50µm sections: Free floating techniques
- Protein gene product 9.5 / neuronal marker
- Epidermal nerve fibers quantified in 4 serial sections/biopsy
- Established nerve counting rules.
- Double stained immuno staining-confocal microscopy
- Statistical analysis

Dermal Schwann cells (cells/mm$^3$)
P75 (low-affinity and pan-neurotrophin receptor)
• Stereology technique
  • Optical Fractionator
  • Thick sections
  • Continuously aligned unbiased counting frames
  • 3 serial sections

Growth Associated Protein-43 (GAP-43)
• Nerve regeneration marker

Morphometry

Ebenezer et al., Brain. 2011;134:1853-63
Results

Neurophysiology

• The mean distance between the two stimulation points was 4.47 ± 0.32 cm.

• MNCV (Mean ± SD): naïve animals was 55.33 ± 7.99 m/s; M.leprae: 54.5 ± 15.4 m/s

• Using a standard threshold of 49 m/s, 3 infected armadillos showed abnormal MNCV in both limbs and 2 animals in at least one limb.

• Depressed CMAP amplitude (<0.9mV) in five infected armadillos
Human: Length dependent innervation

SFN: Progressive decrease of ENF density in distal limb

M. leprae infected armadillos

- ENF density trended lower (no. of ENF/mm) (Mean + SD; Naive: 31±7.1; M. leprae: 26±7.3; p=0.2)
  - 5th percentile: 21/mm  M. leprae: 13/mm - 1 animal

- Quantification ENF is feasible
Schwann cells in dermal cutaneous nerves - Armadillos

Schwann cells - p75
Axons - PGP 9.5

Schwann cells - p75
Axons - PGP 9.5

Schwann cells ensheath axons and co-localize.
**M. leprae infected armadillos**

- Schwann cells trended towards an increasing density
- Mean + SD; Naive: 2285+589; *M. leprae*: 3139+1251; p=0.1
- Dermal Schwann cells harbor and provide a fertile milieu
GAP43 +ve regenerating fibers in nerve bundles co-localize with PGP9.5 +ve axons

- Regenerating fibers are + in *M. leprae* infected armadillos
CONCLUSIONS

- Infected armadillos showed NCV abnormalities compared to naïve animals.
- Similar to human, Naïve Armadillos exhibit length dependent, progressively decreasing epidermal nerve fiber innervation.
- ENF density trended lower among *M. leprae* infected animals – degeneration of small sensory fibers.
- Increasing proliferation of Schwann cells in *M. leprae* infected Armadillos:
  - Small fiber neuropathy in Human–degeneration of Schwann cells
  - *M. leprae* - housing and modify Remak Schwann cells of small sensory fibers in skin
- Corroborates another study

Quantifying ENF density and Schwann cells is feasible in nine-banded armadillos

An important tool for investigating experimental neuropathy and early sensory neuropathy in leprosy:

Diagnostic tool:
- Somatic and autonomic nerve function (sweat gland innervation)
- Pain markers

Progression of disease

Drug trials
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