Contribution of the experimental in vivo models in the comprehension of the leptospirosis pathophysiology

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Leptospirosis, a complex zoonosis

Polymorphism in human
+/- symptomatic
Flu-like symptoms
Weil’s syndrome (10%†)
Pulmonary hemorrhages SPHS (50%†)

Asymptomatic reservoirs
Especially rodents
Rats and mice

Other (+/- susceptible) mammals
Domestic and wild animals
Depending on infecting strain

in vivo experimental models used for the comprehension of the leptospirosis pathophysiology

Ko, Goarant & Picardeau, Nat Rev Microbiol 2009
Rats and mice: chronic carriers of Leptospira

Detection and lesions in kidneys
Related to interstitial nephritis
Variability depending on mouse strain

Resistant and asymptomatic animals: not useful to study the severe symptoms of leptospirosis...
Susceptible models of leptospirosis

**Golden Syrian hamster**
Peritoneal injection

**Restoring virulence** *(Leptospira culture)*
Characterization of strain infectivity
Lethal Dose 50% *(LD$_{50}$)* and 100% *(LD$_{100}$)*
Evaluation of potential vaccines

Examining pathology

**Disadvantages**
Genome not sequence
No serological kit available

**Also guinea pigs and gerbils**
Strain virulence and vaccine efficacy

Infected hamsters used to reproduce severe lesions observed in human cases *(multiple organ failures)*

*Mesocricetus auratus (†)*
Haake, Curr Protoc Microbiol 2006

*Cavia porcellus (†)*

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Hamster (1/3): pulmonary failures

Control hamster

Infected hamster (day 2 pi)

Infected hamster (day 4.5 pi, †)

Normal alveolar architecture
(HES X200)

Edema and hemorrhages
(HES X200)

Large foci of hemorrhages
(HES X100)
Hamster (2/3): renal and hepatic dysfunction

Icteric tissue and blood in the bladder (arrow) (infected, day 4 pi)

Control hamster
Normal hepatic structures (HES X200)

Infected hamster (day 2 pi)
Necrotic hepatocytes (HES X400)

Infected hamster (day 4.5 pi, †)
Inflammation of portal vein (HES X400)

Normal glomeruli and renal tubules (control, HES X200)

Hemorrhage in renal tubules (day 4.5 pi, †, HES X100)
Hamster (3/3): characterization of virulence

Several Brazilian isolates

(I) To test isolate virulence
(II) To standardize LD$_{50}$

Variability of virulence (lethality for 5/7 isolates)
Highly virulent strains (< 200 Leptospira)

Zuerner et al., Vet Path 2012

<table>
<thead>
<tr>
<th>Table 1. Microscopic Analysis of Steiner-Steiner-Stained Sections: Tissue Distribution of Serovar Hardjo Strains</th>
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</thead>
<tbody>
<tr>
<td>Section</td>
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<tr>
<td>Bladder</td>
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<td>Spleen</td>
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<td>Uterus</td>
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</table>

Silva et al., Vaccine 2008

Detection in hamster organs

Two different Hardjo strains

Chronic carriage for one strain

≠ strains used in laboratory

Verdun (LD$_{50} = 1.10^8$) vs. Fiocruz (LD$_{50} <200$)

Characterization is primordial

Disparity of virulence (LD$_{50}$) but also variability in tissue lesions and colonization depending on strains
Pathogen dissemination and bacterial load in susceptible animal models

Detection in hamster (†) organs (full bars)

Liver (qPCR, Wharthin-Starry)

Lungs (qPCR)

Kidneys (Wharthin-Starry)

Grey: liver
Black: kidneys
White: lungs

Bacterial load in guinea pig (†) organs

Kinetic (qPCR) – Fiocruz strain
Low level of bacteria load in lungs

Difference in bacterial load depending on organs
Pathogenesis in organs failures?

Matsui et al., Infect Immun 2011

Lourdault et al., J Med Microbiol 2009
Pathogenesis during acute infection in guinea pigs

Various mechanisms involved in the pathogenesis in susceptible hosts: role of immune system?

Guinea pig (†) lungs
Ig and C3 complement deposit

<table>
<thead>
<tr>
<th>Guinea pig no.</th>
<th>Strain, dose</th>
<th>Day of sacrifice</th>
<th>IF IgM</th>
<th>IF IgG</th>
<th>IF IgA</th>
<th>IF C3</th>
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Nally et al., Am J Pathol 2004

Merien et al., FEMS Microbiol Lett 1998

Guinea pig (†) liver
Hepatocyte apoptosis
Pic of apoptosis at 48h pi
Comparative studies between models: cytokine regulation

Hamster (†) vs. mouse

Early regulation of pro-inflammatory response in mice

Vernel-Pauillac & Goarant, Plos NTD 2010

Cytokine expression in hamster blood (Day 3 pi)
Survival vs. lethality
Lower expression level for survival

Matsui et al., Infect Immun 2011

Trouble in the cytokine balance: maintained in hamster (†) while rapidly restored in mouse or survival
Use of genetically modified mouse models

**Mouse deficient in Toll-like receptor (TLR)**

Lethality in TLR4 KO mouse and DKO TLR2 and TLR4 important in the survival

**Mouse deficient in cytokine (IL-4, IFNγ) or receptor (TNF-α)**

Survival but ↑ lesions for TNFR-KO mouse

Importance of TNF-α receptor in the early control of infection

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**Contribution of these models in the discover of atypical Leptospira recognition receptors** (mouse TLR2/TLR4, human TLR2)

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Athanazio et al., Act Tropica 2008

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Nephritis ++</th>
<th>Nephritis +</th>
<th>None</th>
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<td>5/8</td>
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<td>8/15</td>
<td>5/15</td>
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<td>BALB/c IL4-KO</td>
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<td>BALB/c wild-type</td>
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Chassin et al., J Immunol 2009

[Graph showing cumulative survival]
Pathogen adaptation in vivo depending on infected host

Leptospira gene expression in blood

Hamster (†) vs. mouse
LipL32, major protein, binding TLR2
Lower expression level for mice
→ Evasion strategy from host immunity?

Shift in the LPS-O-Ag production in kidneys
Guinea pig (†) vs. rat
Shift related to virulence in host

Nally et al., Plos Infect Immun

Matsui et al., Appl Environ Microbiol 2012

Utility of in vivo models used in comparative study for a better comprehension of leptospirosis pathophysiology

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Acknowledgements

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Thank you for your attention!