Using wild mice to tackle genetic control of resistance to infectious diseases (West-Nile, Plague, Rift Valley Fever)
1 – Two complementary approaches to identify genes controlling resistance to infectious diseases relevant for the human and animal species.

2 – Classical laboratory inbred strains lack genetic and phenotypic variation.

3 – Analyzing wild-derived mouse strains can be key to success (3 examples).

4 – The Collaborative Cross as the genetic reference population for this decade (and more...).
Reverse genetic approach

Genetic difference → Phenotypic difference

resistant

Random mutations

Targeted mutations

Bruce Beutler
Nobel 2011

susceptible

Identify mutation
Forward genetic approach

Phenotypic difference → Genetic difference

resistant

×

susceptible

+ genotyping

C → R

T → S

Causal polymorphism
## Forward genetic approach

<table>
<thead>
<tr>
<th>Pathogen(s)</th>
<th>Locus (Gene)</th>
<th>Main cell type(s)</th>
<th>Protein function or biological process/mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td><em>Ity-Lsh-Bcg</em> (N rampage1)</td>
<td>M</td>
<td>Iron transporter/regulation of intraphagosomal iron</td>
</tr>
<tr>
<td><em>Leishmania donovani</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium bovis</em> (BCG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td><em>Lps</em> (Tlr4)</td>
<td>M</td>
<td>Surface receptor for bacterial LPS/cellular recognition of LPS</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td><em>C5</em> (C5a)</td>
<td>unknown</td>
<td>Component of complement cascade/proinflammatory activity</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plasmodium chabaudi AS</em></td>
<td><em>Char4</em> (Pkkr)</td>
<td>E</td>
<td>Pyruvate kinase/glycolysis in erythrocytes (role for ATP production)</td>
</tr>
<tr>
<td><em>Plasmodium chabaudi AS</em></td>
<td><em>Char9</em> (Vnn1/Vnn3)</td>
<td>E</td>
<td>Pantetheinases/production of the antioxidant cysteamine</td>
</tr>
<tr>
<td>Vesicular stomatitis virus (VSV)</td>
<td><em>Lps2</em> (Trif&lt;sup&gt;-&lt;/sup&gt;)</td>
<td>M</td>
<td>Toll-receptor–associated activator of IFN/regulation of the Tlr3- and Tlr4-dependent signaling pathway</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus (MCMV)</td>
<td><em>Cpg1</em> (Tlr9)</td>
<td>M</td>
<td>Transmembrane receptor/recognition of pathogen-derived molecules</td>
</tr>
<tr>
<td>Cytomegalovirus (MCMV)</td>
<td><em>Jinx</em> (Unc13d)</td>
<td>NK, CTL</td>
<td>Membrane trafficking/priming (fusion) of cytoplasmic vesicles</td>
</tr>
</tbody>
</table>

Origin of laboratory mice

- **M. m. domesticus**: 92%
- **M. m. musculus**: 7%
- **M. m. molossinus**: 1%

<table>
<thead>
<tr>
<th></th>
<th>B6</th>
<th>DBA/2</th>
<th>A</th>
<th>BALB/C</th>
<th>C3H</th>
<th>AKR</th>
<th>129S1</th>
<th>NZW</th>
<th>FVB</th>
<th>NOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. m. domesticus</td>
<td>0.92</td>
<td>0.91</td>
<td>0.94</td>
<td>0.95</td>
<td>0.92</td>
<td>0.94</td>
<td>0.91</td>
<td>0.87</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td>M. m. musculus</td>
<td>0.07</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
<td>0.07</td>
<td>0.05</td>
<td>0.08</td>
<td>0.11</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>M. m. castaneus</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Yang et al., Nat. Genet., 2007
Wild-derived inbred strains

- **M. m. domesticus**
  - WLA/Pas, WMP/Pas
  - WSB/Ei

- **M. m. musculus**
  - MBT/Pas, PWK/Pas,
  - PWD/Ph

- **M. m. molossinus**
  - MOLF/Ei

- **M. m. castaneus**
  - CAST/Ei

- **M. spretus**
  - SEG/Pas, STF/Pas,
  - SPRET/Ei
### West Nile virus

Flaviruses are positive-sense, single-stranded RNA viruses

### Phylogenetic relationships of flaviviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Serocomplex</th>
<th>Clade</th>
<th>Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Nile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kunjin</td>
<td>None</td>
<td>VI</td>
<td></td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murray Valley encephalitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St Louis encephalitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue-1</td>
<td>Dengue</td>
<td>IX</td>
<td>Mosquito-borne</td>
</tr>
<tr>
<td>Dengue-3</td>
<td>None</td>
<td>VII</td>
<td></td>
</tr>
<tr>
<td>Dengue-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>None</td>
<td>VII</td>
<td></td>
</tr>
<tr>
<td>Central European encephalitis</td>
<td></td>
<td>IV</td>
<td>Tick-borne</td>
</tr>
<tr>
<td>Far Eastern encephalitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powassan</td>
<td>None</td>
<td>III</td>
<td>No vector</td>
</tr>
<tr>
<td>Dakar bat</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Meningo-encephalitis syndromes**
WN virus spreading in the USA

• 515 annual cases in USA (2009) CDC, Atlanta.
  278 (54%) West Nile meningitis or encephalitis (neuroinvasive disease),
  222 (43%) West Nile fever (milder disease)
WN virus cycle(s)

Transovarian sexual routes

Mosquito vectors *Culex*

Vertebrate reservoirs

Dead-end hosts
**WN Neuroinvasive Disease (WNND)**

**Mild illness** *(West Nile fever)*
~ 20% among infected individuals

**Clinical severity**
< 1% of infected individuals develop severe neurological disease

**Case-fatality rate**
~ 5-20% among hospitalized patients during 2002-2003 outbreaks
~ 10% among patients with meningo-encephalitis

This suggests the existence of genetics factors controlling the susceptibility of the host
### WNV susceptibility in mice

<table>
<thead>
<tr>
<th>Mouse strains*</th>
<th>Mortality(^\d), no. of dead/no. of infected mice</th>
<th>Days of death (extremes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>6/6</td>
<td>8–10</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>6/6</td>
<td>7–10</td>
</tr>
<tr>
<td>MAI/Pas</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>MBT/Pas</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>(C57BL/6 × MAI)F1</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>(C57BL/6 × MBT)F1</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>(BALB/c × MAI)F1</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>(BALB/c × MBT)F1</td>
<td>0/6</td>
<td>NA</td>
</tr>
<tr>
<td>(C57BL/6 × MAI)F1 × C57BL/6</td>
<td>16/42</td>
<td>7–13</td>
</tr>
<tr>
<td>(C57BL/6 × MBT)F1 × C57BL/6</td>
<td>8/25</td>
<td>10–12</td>
</tr>
<tr>
<td>(BALB/c × MAI)F1 × BALB/c</td>
<td>19/55</td>
<td>7–13</td>
</tr>
<tr>
<td>(BALB/c × MAI)F1 × BALB/c</td>
<td>31/81</td>
<td>7–12</td>
</tr>
</tbody>
</table>

Dominant resistance allele

*Mashimo et al., PNAS, 2002*
203 [(MBT/Pas x BALB/c)F1 x BALB/c]BC1 progeny
The mapping was done using only the 74 BC1 mice that died.

The critical interval was further refined to 0.4 cM by genotyping additional recombinants

Mashimo et al., PNAS, 2002
**Gene map of the critical region**

**20 genes /ESTs:**
- *FLN29*, gene with ligase activity
- *Rbm19*, RNA binding motif
- *RPL6*, ribosomal protein L6
- *PTN11*, Tyrosine phosphatase
- *Rabphilin 3A*
- *Rasal1*, RAS protein activator like 1
- *Tpcn1*, calcium ion transport

**Oligo-adenylate synthetase genes:**
- *Oas 1a* to *Oas1h*, eight genes
- *Oas2, Oas3*
- *Deltex*
- *SDS*, Serine dehydratase
- *LHX5*, LIM homeobox protein

*Mashimo et al., PNAS, 2002*
Non-sense mutation in *Oas1b* gene

**Stop codon**

- **BALB/c**
  - GGGCTTCTGAACCGGT

**Arginine**

- **MBT/Pas**
  - GGGCTTCCGAACCGTC

**Laboratory mice (susceptible)**

- **C57BL/6**
  - GGGCTTCTGAACCGGT

- **C3H/HeJ**
  - GGGCTTCTGAACCGGT

- **129/Sv**
  - GGGCTTCTGAACCGGT

- **DBA/2J**
  - GGGCTTCTGAACCGGT

- **DDK/Pas**
  - GGGCTTCTGAACCGGT

- **NZB/Ola**
  - GGGCTTCTGAACCGGT

- **NZW/Ola**
  - GGGCTTCTGAACCGGT

**Wild mice (resistant)**

**Mus m musculus**

- **MAI/Pas**
  - GGGCTTCCGAACCGTC

- **PWK/Pas**
  - GGGCTTCCGAACCGTC

**Mus m domesticus**

- **WMP/Pas**
  - GGGCTTCCGAACCGTC

**Mus spretus**

- **SEG/Pas**
  - GGGCTTCCGAACCGTC

- **STF/Pas**
  - GGGCTTCCGAACCGTC

**Laboratory mice (resistant)**

- **PL/J**
  - GGGCTTCCGAACCGTC

*Mashimo et al., PNAS, 2002*
Transgenic complementation

CMV/chicken b-actin promoter

CAG

Oas1b cDNA

pA SV40

BALB/c genetic background

Survival curve

Percent survival

Days after infection

BALB/c Tg/+

BALB/c +/-

Simon et al. 2011 Virology, in press
Relevance for other species

Genetic Variation in *OAS1* Is a Risk Factor for Initial Infection with West Nile Virus in Man

Jean K. Lim¹, Andrea Lisco², David H. McDermott¹, Linda Huynh¹, Jerrold M. Ward³, Bernard Johnson⁴, Hope Johnson⁴, John Pape⁵, Gregory A. Foster⁶, David Krysztof⁶, Dean Follmann⁷, Susan L. Stramer⁶, Leonid B. Margolis², Philip M. Murphy¹*

February 2009 | Volume 5 | Issue 2 | e1000321

*OAS1* Polymorphisms Are Associated with Susceptibility to West Nile Encephalitis in Horses

Jonathan J. Rios¹, JoAnn G. W. Fleming², Uneeda K. Bryant³, Craig N. Carter³, John C. Huber, Jr.⁴, Maureen T. Long⁵, Thomas E. Spencer², David L. Adelson⁶*

¹ McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, Texas, United States of America, ²Department of Animal Science, Texas A&M University, College Station, Texas, United States of America, ³Livestock Disease Diagnostic Center, University of Kentucky, Lexington, Kentucky, United States of America, ⁴School of Rural Public Health, Texas A&M University, College Station, Texas, United States of America, ⁵College of Veterinary Medicine, University of Florida, Gainesville, Florida, United States of America, ⁶School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, South Australia, Australia

May 2010 | Volume 5 | Issue 5 | e10537
Yersinia pestis and plague

Yersinia pestis

- Gram-negative, enterobacteria
- infects wild rodents (>200 species)
- transmitted through flea bites
- causes plague epidemics in humans

Alexandre Yersin
(1863-1943)
identified the bacteria responsible for plague, during the Hong-Kong epidemics (1894)

Paul-Louis Simond
(1858-1947)
demonstrated the transmission of the bacteria by rat fleas (1897)

Alexandre Yersin in front of his "laboratory"
The plague disease

Bubonic form
- inflammation of the lymph node draining the bite site: "bubo"
- intense replication of bacteria
- evolves to suppuration and dissemination to other lymph nodes

Septicaemic form
- following massive or IV infection
- usual signs of septicaemia with septic shock

Pneumonic form
- the most dangerous and fatal form
- secondary to septicaemic or by inhalation
- fulminant haemorrhagic pneumonia
Human plague

Black death

- spread from Asia to Europe ~1350
- 20 M people died (1/3 of Europe)
- Paris, Venice, etc… : > 50% died

Plague epidemics

- 200 M in human history
- >2,000 cases reported each year (99% in Africa) – 200 deaths
- coincide with foci of infected rodents
Cats, rats, mice, rabbits, camels, turkeys, and monkeys are highly susceptible to plague.

Dogs and cows are resistant.

In humans, not all exposed individuals become sick or die.

"Not all of them died, but all were struck"
• Laboratory strains are highly susceptible to fully virulent *Y. pestis* infection.

• Differences in susceptibility can be observed only when using *Y.p.* strains lacking virulence factors.
SEG/Pas mice are exceptionnaly resistant to Y. p. 

100 cfu, strain C092, s.c., 12-15 wks

Blanchet et al., Genes and Immunity, 2010
QTL mapping of resistance loci

BSB : (B6 x SEG)F1 x B6

Y. p. challenge : 100 cfu, strain C092, s.c., 12 wks
High-density SNP genotyping (>700 markers)
Three QTLs increase survival by 20%.
Y. p. resistance in SEG: a model
Y. p. resistance in congenic mice
Zoonosis in Africa
Spread to Yemen and Saudi Arabia in 2000
Infectious disease due to an enveloped virus transmitted mainly by mosquitoes (Phlebovirus)

Reasons to study RVF virus

- Human and animal health problem
- No safe vaccine for protection nor antiviral agents for therapy
- Arbovirus transmitted by many species of mosquitoes
- Potential bioterrorism agent

![Diagram of Rift Valley fever virus](image)

- Segment L (6404 nt)
- Segment M (3885 nt)
- Segment S (1690 nt)

90 à 100 nm
Rift Valley fever virus-induced diseases

**RVF in animals**
- Abortions
- High fatalities in young animals
- Necrotic hepatitis with or without haemorrhagic state
- Indigen breeds of African cattle and sheep are resistant

**RVF in humans**
- Usually uncomplicated acute febrile illness but
  - hepatocellular failure
  - acute renal failure
  - hemorrhagic manifestations
  - meningoencephalitis and retinitis
  - death associated with hepatorenal failure, shock, severe anemia

occur in 1% of humans that become infected with Rift Valley fever virus

The different outcomes both in animals and humans suggest the existence of genetic factors controlling the susceptibility of the host.
Survival of various inbred strains of mice following infection by $10^2$ PFU RVF ZH548 i.p.
### Viral load in BALB/c and MBT infected mice

<table>
<thead>
<tr>
<th>D0</th>
<th>D1</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cByJ</td>
<td>0</td>
<td>4.2 ± 0.5</td>
<td>All mice were still alive</td>
</tr>
</tbody>
</table>

- **100 PFU Rift Valley fever virus**
- **log(PFU/ml)**

<table>
<thead>
<tr>
<th>D1</th>
<th>D3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MBT/Pas</td>
<td>3.1 ± 0.3</td>
<td>7.7 ± 0.3</td>
</tr>
</tbody>
</table>

- **100 PFU Rift Valley fever virus**
- **log(PFU/ml)**

→ Earlier and higher viral production in MBT mice than in BALB/c mice.
MBT cells produce higher virus titers than BALB/c cells, at various MOI, suggesting that BALB/c cells are able to limit viral production.

Zaverucha do Valle et al., J. Immunol., 2010
9 hours post-infection

27,000 unique transcripts

Number of differentially expressed genes

Upregulated

BALB/cByJ

MBT/Pas

Differentially regulated genes (twofold cutoff)

Number of genes:

% cellular transcripts:

→ Infection differentially regulates more cellular genes in MBT cells than in BALB/c cells.

Zaverucha do Valle et al., J. Immunol., 2010
229 unique genes differentially regulated following infection with Rift Valley fever virus

87 genes are highly upregulated after infection in BALB/cByJ cells, but moderately or not upregulated in MBT/Pas cells

Zaverucha do Valle et al., J. Immunol., 2010
Genes highly upregulated in MEFs from BALB/c, but not from MBT/Pas mice

- Interferon stimulated gene 20
- Interferon stimulated gene 12
- Interferon stimulated gene 15
- Interferon stimulated gene 56
- Interferon stimulated gene 60
- Interferon induced gene 44
- Interferon induced gene 35
- Member of the P200 family
- p47 GTPase family
- MDA-5 RNA helicase
- RIG-I RNA helicase
- Interferon regulatory factor 7 (IRF7)
- Interferon regulatory factor 9 (IRF9)
- Oligoadenylate synthetase (OAS1a)
- Oligoadenylate synthetase (OAS1b)
- STAT1
- STAT2

Zaverucha do Valle et al., J. Immunol., 2010
→ BALB/c cells display a strong innate immune response to infection with the RVFV
Weak gene activation in MBT

Type I interferon antiviral innate response

Kinetic study by gRT-PCR

→ MBT cells display a weak innate immune response to infection with the RVFV
Gene expression study by RT-PCR

Group 1
Late higher expression in MBT/Pas cells

Expressed in a Stat2-dependent manner

Ifit3

Ifna4

Ifnb1

Group 2
Delayed induction in MBT/Pas cells

Direct target of IRF3

Ifit1

Known sensor of RVFV dsRNA

Rig-I

IFNAR activation pathway

Stat2

Group 3
Low or no induction in MBT/Pas cells

Required for IFN-α4 expression

Irf7

Ubiquitin-like protein that modifies > 150 proteins by ISGylation

Lsg15

Oligoadenylate synthetase

Oasl2
Do the genes whose expression in MBT/Pas cells is low or delayed play a role in limiting viral production?

- Inhibition of RVFV-induced genes by specific siRNA

\[ \text{Isg15 and Oasl2 play functional role in the inhibition of viral production} \]

Zaverucha do Valle et al., J. Immunol., 2010
Mapping resistance loci

→ F2 mice are intermediate between MBT/Pas and BALB/c mice
576 F2 mice were challenged with \(10^2\) PFU RVF virus.

Genotyping with 315 polymorphic markers, 240 SNPs and 75 microsatellites distributed along the whole genome.
Three QTLs affect time to death
QTL mapping and gene expression

Do differentially regulated genes in BALB/c and MBT/Pas MEFs map to the QTLs on chromosome 2, 5 and 11?
Towards an ideal mouse population?

- Inbred strains provide information dependent on their genotype.
- Outbred stocks are not a valuable alternative (lack of genetic variation, irreproducibility, poor genetic characterization).
- Testing several inbred strains is an optimal design but common lab strains are closely related.
Towards an ideal mouse population?

- Include genetic variation present across *M. musculus* subspecies in a novel genetic reference population with ideal features:
  - Maximize genetic variation across a collection of strains
  - Optimize genetic resolution to facilitate gene identification
  - Balanced contribution from each progenitor
  - Detailed characterization of the genotype of each strain
Collaborative Cross design

- These 8 strains capture 89% of the mouse genetic variation (2x that observed across human populations).

- Developed at:
  - University of North Carolina (Chapel Hill),
  - Tel-Aviv University,
  - Perth (Australia).

- 300-400 strains at the completion of the program.

- First 70 strains to be released beginning of 2012.
Collaborative Cross

Data on 184 lines
Collaborative Cross: first data

Collaborative Cross mice and their power to map host susceptibility to Aspergillus fumigatus infection

Caroline Durrant,¹ Hanna Tayem,² Binnaz Yalcin,¹ James Cleak,¹ Leo Goodstadt,¹ Fernando Pardo-Manuel de Villena,³ Richard Mott,¹ and Fuad A. Iraqi²,⁴

*Genome Research* 21:1239–1248 © 2011
Action BM0901 2009 - 2013

SYSGENET: European systems genetics network for the study of complex genetic human diseases using mouse genetic reference populations

Participating countries: AT, CH, CZ, DE, DK, EE, ES, FI, FR, GR, IL, IT, LU, NL, PL, UK
Chair: Klaus Schughart, DE, klaus.schughart@helmoltz-hzi.de

Working Groups:
1) GRP Resources
2) Phenotyping and genotyping
3) Bioinformatics
4) International outreach
5) Training and mobility

- Plans to import at least 75 strains in Europe. To be distributed by TAU.
Conclusions

- There is tremendous variation across mouse strains in susceptibility to infectious diseases.

- Wild-derived inbred strains provide much larger genetic and phenotypic variation than laboratory strains.

- These natural variations are often controlled by multiple genes with moderate effects.

- The Collaborative Cross will provide unprecedented opportunity to discover new mouse models, in all biological fields.
Acknowledgements

Mouse functional genetics unit

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Tania Zaverucha do Valle
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Jean-Jacques Panthier

Yersinia unit

Christian Demeure
Elisabeth Carniel

Molecular Genetics of Bunyaviruses unit

Agnès Billecocq
Michèle Bouloy

Molecular Interactions
Flaviviruses-Host

Klaus Schughart
Rudi Alberts
Marie-Pascale Frenkel
Philippe Desprès