Animal Models in Research

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Why animal models in research?

- Obvious ethical, social, religious prohibitions have prevented human experimentations
- Animal models play a key role in studying human diseases
Why animal models in research?

- Ideas & Trends: Of Mice and Men; Why Test Animals to Cure Human Depression?
- Do mice commit suicide?
Why animal models in research?

- Article in the examiner described suicidal mouse behavior in response to brain Toxoplasmosis
What exactly is an animal model?

• It is like a biological phenomenon that one species has in common with the target species.
• It is like a concept of “analogy.”
• It is NOT a claim of identity with what is being modeled, but like a substitute for the target.
A comprehensive definition

- Wessler’s original definition: “a living organism in which biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon resembles that in humans or other species of animal.”
Categories of Animal Models in Research

- **Induced Model**: Healthy animal in which condition is induced.
- **Spontaneous Model**: Utilize naturally occurring genetic variants.
- **Genetically Modified Model**: Also called transgenic disease models.
- **Orphan Model**: Disease occurring in animal, not described in human.
- **Negative Models**: In which a certain disease does not develop.
Extrapolation from animals to human

• The rationale behind extrapolating results from animals to human is based on the extensive homology and evolutionary similarity between morphological structures and physiological processes among different animal species and between animals and humans.
Extrapolation from animals to human

• What is noxious or ineffective in nonhuman species can be innocuous or effective in humans and vice versa. For example, penicillin is fatal for guinea pigs but well tolerated by humans; aspirin is teratogenic in cats, dogs, guinea pigs, rats, mice, and monkeys but obviously not in pregnant women.
Extrapolation from animals to human

• In general, metabolism or detoxification and excretion of a drug are not directly correlated with body size, but more accurately with the metabolic rate of the animal.
Animal models for Inflammatory disorders

<table>
<thead>
<tr>
<th>Name</th>
<th>Disease</th>
<th>Type</th>
<th>Species</th>
<th>Rationale</th>
<th>Strengths/advantages</th>
<th>Weaknesses/disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant induced arthritis</td>
<td>Inflammation/</td>
<td>Joint destruction</td>
<td>Rat (mouse)</td>
<td>Designed for NSAIDs</td>
<td>Highly reproducible NSAIDs work</td>
<td>Limited predictability for other drug classes</td>
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<tr>
<td></td>
<td>rheumatoid arthritis/pain</td>
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<tr>
<td>Collagen induced arthritis</td>
<td>Rheumatoid arthritis/pain</td>
<td>Joint destruction</td>
<td>Mouse (rat)</td>
<td>Most reflective of human joint pathology</td>
<td>Responds to NSAIDs/TNF inhibitors/IL-1 inhibitors</td>
<td>Not reflective of all human joint pathology-acute disease model, self-limiting, limited predictability for cell signaling based drugs</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Some aspects of joint disease</td>
</tr>
<tr>
<td>Endotoxin induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Joint destruction</td>
<td>Mouse, rat</td>
<td>Inflamed joints</td>
<td>Responds to NSAIDs</td>
<td>Model is acute</td>
</tr>
<tr>
<td>Antibody induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Acute joint destruction</td>
<td>Mouse</td>
<td>Reflects acute phase of disease</td>
<td>Responds to NSAIDs, P38 and FDE inhibitors</td>
<td></td>
</tr>
<tr>
<td>Carrageenan Paw model</td>
<td>Inflammation edema</td>
<td>Inflamed paw</td>
<td>Mouse</td>
<td>Generalized inflammation</td>
<td>IL-1RA, anti-IL-6 NSAIDs model</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Tail flick</td>
<td>Pain</td>
<td>No treatment required</td>
<td>Mouse, rat</td>
<td>Acute pain (burn)</td>
<td>Very specific for analgesics</td>
<td>Does not reflect neuropathic or inflammatory pain</td>
</tr>
<tr>
<td>EAE (EAE)</td>
<td>Multiple Sclerosis</td>
<td>Neural sheath derived</td>
<td>Mouse (guinea pig, rabbit, primates)</td>
<td>Demonstrates relapsing/remitting MS like disease</td>
<td>Can be used with a variety of therapeutics but not always predictively</td>
<td>Generally a self-limiting disease unlike MS</td>
</tr>
<tr>
<td>Endotoxin induced sepsis</td>
<td>Endotoxic shock, septicemia</td>
<td>Primarily acute in susceptible animal species</td>
<td>Mouse, rat, guinea pig, rabbit</td>
<td>Reflects the result of acute bacteremia</td>
<td>No predictive utility</td>
<td>Some mouse strains are resistant-human disease is demonstrably different</td>
</tr>
<tr>
<td>Inhaled antigen induced</td>
<td>Allergic disease asthma</td>
<td>Tracheal inflammation</td>
<td>Mouse, rat, rabbit, dog, monkey</td>
<td>Shows smooth muscle constriction in the trachea and will respond to many anti-asthmatic drugs</td>
<td>Not predictive</td>
<td>No animal exactly mimics human bronchial constriction</td>
</tr>
<tr>
<td>tracheal constriction models</td>
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<tr>
<td>Delayed type hypersensitivity</td>
<td>Skin inflammation allergy</td>
<td>Skin inflammation</td>
<td>Mouse, rat, guinea pig</td>
<td>Shows cellular infiltrate and classic DTH</td>
<td>Useful</td>
<td>Can be used for topical treatments for allergic disease</td>
</tr>
<tr>
<td>models</td>
<td></td>
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<td></td>
<td></td>
<td>Not completely reflective of human disease (gut flora differences between mouse and man)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>IBD, colitis, Crohn's disease</td>
<td>Autoimmune inflammation and bowel destruction</td>
<td>Mouse</td>
<td>Shows most of the features of Crohn's disease and ulcerative colitis</td>
<td>Not always predictive although anti-TNF's and some other drugs work here</td>
<td>Amplifies specific gain or loss effects of specific genes</td>
</tr>
<tr>
<td>(colitis, Crohn's disease)</td>
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<tr>
<td>Transgenic mouse models of</td>
<td>Many autoimmune/</td>
<td>Can show cellular and</td>
<td>Mouse</td>
<td>Allows detailed study of effects of gene depletion or amplification/mutation in vivo</td>
<td>Can uncover new targets for therapeutic evaluation</td>
<td>Not the same physiology as in man</td>
</tr>
<tr>
<td>autoimmune diseases</td>
<td>inflammatory diseases</td>
<td>tissue features that</td>
<td></td>
<td></td>
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<tr>
<td>Humanized mouse models</td>
<td>Primarily used for</td>
<td>resemble diseases</td>
<td>Mouse</td>
<td>Used to probe specific aspects of human immunity may be studies in normal volunteers (e.g. HIV studies)</td>
<td>Some aspects of human immunity may be studies in an in vivo setting</td>
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<tr>
<td></td>
<td>hematologic studies of</td>
<td>under study</td>
<td></td>
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<tr>
<td></td>
<td>various types</td>
<td>Human immune cells can be studied in vivo reflecting some aspects of human immunity</td>
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</tbody>
</table>
Controversies

- Seok et al. reported that mouse models poorly mimic human inflammatory diseases, in terms of gene expression.
## Animal models of cancer

### Characteristics and applications of classes of animal models of cancer and their utility in oncology drug discovery.

<table>
<thead>
<tr>
<th>Type of cancer model</th>
<th>Species</th>
<th>Primary features</th>
<th>Utility</th>
<th>Strengths and advantages</th>
<th>Weaknesses and caveats</th>
</tr>
</thead>
</table>
| Sc ir, ectopic xenograft models with established tumor lines | Immunocompetent and immuno-deficient rodents | - Ectopic implantation of cell lines or tissue fragments  
- Non-physiologic growth location | - Facile and rapid pharmacological screening of NCEs throughout discovery screens  
- Tumor PK/PD relationships  
- Anti-tumor efficacy | - Reproducible  
- Cost and time effective tumor measurements  
- Applicable to many tumor cell types  
- Assess influences of immune surveillance and evasion (immuno-competent hosts) | - Limited histological and phenotypic similarities to primary cancers  
- Loss of tumor heterogeneity  
- Low metastatic rates  
- Lack of native tumor microenvironment |
| Orthotopic tumor models with established tumor lines | Immunocompetent and immuno-deficient rodents | - Implantation into organ of origin  
- Reconstitutes organ micro-environment  
- Local and metastatic spread | - Mid-later stage discovery screening  
- Assess survival endpoints  
- Evaluate effects on primary tumor growth in proper microenvironment  
- Assess influence on local and metastatic tumor spread | - High metastatic rates  
- Correct tumor microenvironment  
- Assessment of tumor-stromal interactions  
- Assess anti-tumor efficacy of primary and metastases  
- Assess influence on immune surveillance and evasion (immuno-competent hosts) | - More time and labor intensive  
- In vivo artificial selection of cell lines  
- Histological dissimilarities with human tumors  
- Loss of tumor heterogeneity  
- Imaging modalities needed for in situ assessment of tumor development |
| Transgenic and GEMM tumor models | Immunocompetent mice | - Spontaneous and autochthonous development of tumors in appropriate micro-environment in situ | - Mid-later stage discovery screening  
- Evaluate effects on immune surveillance, invasion and metastasis, and tumor angiogenesis | - Phenotypic, histological and genetic similarities to primary cancers  
- Models human cancer under more physiological conditions  
- Assess influences on tumor immune surveillance and evasion | - Asynchronous development of tumors  
- Outbred mice with non-uniform genetic backgrounds  
- More time and labor intensive  
- Metastatic patterns may not mimic human cancers  
- Lack of tissue-specific promoters  
- Imaging modalities needed for in situ assessment of tumor development |
| Primary human tumorgraft models | Immuno-deficient mice | - Direct implantation/propagation of freshly excised human tumors | - Mid-later stage discovery screening  
- Evaluate HCE effects on original human tumor ectopically or orthotypically | - Preserves and stabilizes genetic, histological, and phenotypic features primary tumor  
- Maintains stromal and stem cell components of primary tumor  
- Facilitates biomarker assessment  
- Potential to metastasize  
- Ease of tumor measurements | - Access to freshly excised human tumors  
- High front-end costs and labor-intensive preparation  
- Limited engraftment rates and long latency for tumor development |
| Carcinogen-promoter-induced multi-stage tumor models | Immunocompetent rodents (mice, rats, hamsters) | - Time-dependent, multi-stage progression of tumor development in response to carcinogens and tumor-promoting agents  
- Etioologically relevant environmental carcinogens induce tumors in organ-specific manner | - Later stage discovery screens  
- Determine most sensitive stages of tumor development for chemo-preventive and or therapeutic intervention | - Histological, biochemical and phenotypic similarities to human cancers  
- Mimic stage-specific developmental sequence of cancers  
- Reproducible and high penetrance of organ-specific lesions  
- Metastasize to specific sites  
- Assess influences on tumor immune surveillance/evasion | - Long time frames (5-50 weeks) for tumor development  
- High costs in animal maintenance/core  
- Repeated use of carcinogenic agents  
- Outbred mice with non-uniform genetic backgrounds and varied sensitivity to carcinogens  
- Imaging modalities needed for in situ assessment of tumor development (non-skin lesions) |
Animal models for CNS disorders

• Multiple factors determine the choice of animal model for CNS disorders.
  – For studies on the neuropathology, primate and swine model with appropriate gyrations and structural complexities similar to human brain are more suitable.
Animal models of neuropsychiatric disorders

• Despite the challenges, significant progress has been made in the development and optimization of behavioral models for the majority of CNS disorders and these models have provided valuable insights regarding mechanism and treatment when used appropriately.
Animal models of neurodegenerative disorders

• Tremendous effort focused on the development and characterization of animal models for Alzheimer’s disease.

• 3 general categories –
  – pharmacological,
  – lesioned
  – transgenic
Animal models of Pain

• Chronic pain -
  – affects over 25% of the general population
  – medications to treat it have limitations in terms of efficacy and safety.
• Current animal models instrumental in improving understanding of chronic pain and therapies
• Efforts to develop robust and predictive models face numerous challenges
Animal models of Stroke

• Most challenging neurologic disorder to model due to
  – Variable causes
  – Variable consequences
• MCA Occlusion model
Animal models of HIE

- HIE occurs following birth asphyxia and cardiac arrest
- Vanucci model - exposure to low FiO2 and carotid artery occlusion
Piglet model of brain injury following hypoxic-asphyxycar cardiac arrest

- Hypoxia through exposure to 10% FiO2 and
- Asphyxia through ETT clamping
- Resuscitation followed by recovery
Neuroinflammation in a swine model of pediatric cardiac arrest

3-5 day old piglet
- Administration of General Anesthesia
- Endotracheal Intubation and vascular cut-down for Arterial and Venous line placement

Hypoxic-Asphyxic Cardiac Arrest
- Administration of 10% FiO2 for 45 minutes
- Induction of asphyxia through clamping of ETT for 7 minutes
- Chest Compressions and administration of Epinephrine
- Post-arrest care, extubation once neurologic recovery

Perfusion to isolate brain
- Immunohistochemistry staining of brain sections using Anti IBA-1 antibodies

Analysis of IHC stained brain sections
- Unbiased Stereology
- Neurolucida

Utpal Bhalala, MD
Raymond Koehler, PhD
Lee Martin, PhD
Sujatha Kannan, MD

12Hr Sham
12Hr Arrest
12Hr Sham
12Hr Arrest
Piglet model of neuroinflammation following cardiac arrest
Piglet model of neuroinflammation following cardiac arrest
Piglet model of neuroinflammation following cardiac arrest

Neuroinflammation in Selective Vulnerable Areas

Average Volume of IBA-1 positive cells (mm³)

<table>
<thead>
<tr>
<th></th>
<th>Caudate</th>
<th>Putamen</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injured</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
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<td></td>
<td></td>
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<tr>
<td>0.5day recovery</td>
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<tr>
<td>1day recovery</td>
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<td>2day recovery</td>
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<tr>
<td>3day recovery</td>
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<td></td>
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<td>7day recovery</td>
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</tbody>
</table>

Distribution of IBA-1 positive inflammatory cells in injured (N=10) and sham (N=5) animals
Piglet model of neuroinflammation following cardiac arrest

Distribution of viable neurons in injured (N=10) and sham (N=5) animals

- Caudate
- Putamen
- SMC
Piglet neuron profile following cardiac arrest
Why piglet model of cardiac arrest to study neuroinflammation?

• Anatomic and physiologic profile of piglet model similar to human

• Cardiac arrest induces neuro and systemic inflammation
What Next?

• Once we study neuroinflammation in relation to the neuronal death, we plan to study
  – Neuroprotective effects of anti-inflammatory
  – Neuronal-glial cross-talk as a contributor of persistent neuroinflammation
Thank You