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***Immune Relevant Animal Models: Opportunities and Challenges***

**Jungersen and Piedrahita. Introduction: Immune Relevant Animal Models: Opportunities and Challenges, pp. 209-210**

Domain 3; TT3.3

SUMMARY: Animal models are required for immunology studies since the immune system is a highly complex network.  There are large numbers of cell, transcription factors, and genetic pathways involved in immune-mediated effectors.   Mice are one of the main animal models used, but dogs have been studied as well.  Science named treating cancer with a patient’s own immune system “breakthrough of the year” in 2013.  Chimeric antigen receptor (CAR)-T cells are another promising immune treatment of cancers and chronic viral infections.  Xenotransplantation failures are generally due to immune system rejection.  Pigs and nonhuman primates have been used in stem cell research in the field of reverse xenotransplantation, where human stem cells are transplanted in a pig for development before being transplanted back into the human donor.  Vaccine development research relies on the adaptive immune response.  Animal models are important in infectious agent and innate and adaptive host immune responses.  Pigs have been used over ferrets and mice for influenza A research due to their high target and face validity being naturally infected with the same subtypes and display similar clinical symptoms.  The eye, brain, and uterus in pregnancy are considered immune-privileged.  The eye is studied in dry eye and uveitis since ocular tolerance is lost.

QUESTIONS

1. What is reverse xenotransplantation?
2. Why are pigs used over ferrets and mice for influenza A research?
3. What are considered 3 immune privileged sites in mammals?

ANSWERS

1. Human stem cells are transplanted in a pig for development before being transplanted back into the human donor
2. Pigs have high target and face validity being naturally infected with the same subtypes and display similar clinical symptoms
3. Eye, brain, and uterus in pregnancy

**Radaelli et al.** [**Immune Relevant and Immune Deficient Mice: Options and Opportunities in Translational Research**](https://academic.oup.com/ilarjournal/article/59/3/211/5518888)**, pp. 211-246**

Primary Species: Mouse (Mus musculus)

Domain 3: Research

SUMMARY

Introduction: There are many advantages to using mice in biomedical research, however there is ongoing controversy about the translatability and reproducibility of mouse models of disease. While a perfect model of disease relevant to all humans is probably impossible, it is possible to design experiments to address specific research questions using relevant and translational models (including mice). There are many factors that contribute to study outcomes and that can affect reproducibility, including genetic diversity, microbiome, husbandry practices and environment, and experimental inventions. This review article seeks to gather resources and references pertaining to key concepts of mouse immunology, so as to provide relevant information in one place for investigators or others looking to become more familiar with immune diversity in mice. There are several tables included in the review article that are extremely useful but which cannot be reproduced in this summary. Therefore, it is recommended that the reader access the full article to gain access to the tables.

Mouse Nomenclature: Rules for standard nomenclature of mice genes, strains, and substrains have been in use for over 50 years. Accurate mouse nomenclature is essential to scientific communication and reporting of animal research. Early information included guidelines for gene and strain nomenclature, lists of strains and substrains, and lists of abbreviations for the researchers or institutions maintaining the mice. The list of institutions became the “lab codes” and are currently curated by ILAR: <http://dels.nas.edu/global/ilar/lab-codes>. Current gene nomenclature rules for mice (International Committee on Standardized Genetic Nomenclature for Mice) are available online at: <http://www.informatics.jax.org/mgihome/nomen/strains.shtml>. Information rats is available through the Rat Gene Nomenclature Committee at <https://rgd.mcw.edu/nomen/nomen.shtml>, and for human genes through HUGO Gene Nomenclature Committee at <http://www.genenames.org/>. The Mouse Genome Informatics Nomenclature site provides guidance and tutorials for mouse strains, genes, alleles, and mutations and is available at <http://www.informatics.jax.org/mgihome/nomen/gene.shtml>.

**I**nbred Mouse Strains: Immune Relevant Genotypes and Phenotypes: Common inbred mouse strains are genetically well-characterized, and it is recognized that different strains have varying susceptibility to infections, diseases, and tumor development. There are many different well-characterized immune relevant variations among common immune sufficient inbred mouse strains. The genetic diversity among these inbred strains can be due to genetic variations including polymorphic alleles, unique quantitative trait loci (QTL) intervals, or specific haplotypes. The influence of the inbred genetic background can influence many experimental contexts and is crucial knowledge for researchers when developing studies. This information is summarized in Table 1 and Supplementary Table 1 of the article. Even substrains with similar names may have important genetic variations. Table 2 summarizes some immune relevant genetic variations among C57BL/6 substrains. For example, C57BL/6J mice have a missense, loss of function mutation of the *Nlrp12*gene, which primarily controls neutrophil chemotaxis in response to bacterial invasion. As a result, they are more susceptible to certain bacterial infections compared to other C57BL/6 substrains with the wild type *Nlrp12*allele. The *Dock2* mutation in C57BL/6NHsd mice is an example of a mutation that arose in a substrain (of the same name) with colonies maintained at different sites. Mice with the *Dock2Hsd*mutation have reduced splenic marginal zone B cells and increased numbers of CD8+ T cells compared to other C57BL/6N mice. Genetic quality assurance testing and breeding strategies to minimize effects of random mutation and genetic drift are warranted. It is also important to consider the influence of the background strain on spontaneous or genetically engineered mice (GEM), as this can affect research results. When spontaneous or experimentally induced mutations are transferred congenically from the line of origin onto a different inbred background strain, penetrance and expressivity of the phenotype may be positively or negatively affected. The authors illustrate this point by showing that an internet search for immunodeficient mice with the *Prkdcscid* (*scid*) or *Foxn1nu*(nude) mutations reveals more than 20 strains of each on either inbred or non-inbred backgrounds, each with immune variations relevant to their genetic backgrounds. Leakiness, or the tendency of *scid* mice to produce some functional B and T cells as they age, can vary in *scid* mice depending on the background strain. Leakiness is greater on the C57BL/6 and BALB/c backgrounds, low on the C3H/HeJ background, and very low on the nonobese diabetic (NOD) background. Strains susceptible to developing spontaneous autoimmune diseases such as immune-mediated (Type 1-like) diabetes and systemic lupus erythematosus (SLE) or experimentally induced autoimmune conditions, such as experimental autoimmune  encephalitis (EAE) or collagen-induced arthritis (CIA) can be greatly impacted by the immune variations of the background strain. There are several recognized genetic and phenotypic variations among the NOD substrains. Mutations such as *Faslpr*and *Yaa* in mice are associated with the development of spontaneous lupus-like conditions. Inbred strains known to spontaneously develop lupus-like conditions include MRL/MpJ, BXSB/MpJ, NZB, NZW, NZBWF1 (aka NZB/W), NZM2410, and Palmerston North (PN/nBSwUmabJ). MRL/MpJ mice are autoimmune-prone and spontaneously develop an autoimmune phenotype as they age.

* The substrain MRL/MpJ-*Faslpr* have a spontaneous mutation on the *Faslpr*gene, leading to a shorter lifespan and the development of signs of autoimmunity (lymphoproliferative disease, immune complex glomerulonephritis, lupus-like skin disease, arthritis, and vasculitis) much earlier in life than the parent strain. The onset and severity of symptoms associated with *Faslpr*mutation is strain dependent. A more severe lymphoproliferative disease results from the *Faslpr*mutation on the MRL/MpJ strain than one the C57BL/6J background, but is less severe than that seen on the C3H/HeJ background. Immune complex pathologies, however, are more severe and initiate earlier with the *Faslpr*mutation on the MRL/MpJ background than on either the C57BL/6J or C3H/HeJ backgrounds. The MRL/MpJ strain harbors diverse polymorphic alleles, unique QTL, and specific haplotypes that contribute to this strain’s susceptibility to autoimmune diseases.
* BXSB/Mp mice are a recombinant inbred strain developed by crossing a C57BL/6J female with an SB/Le male. These mice develop a lupus-like disorder that is accelerated in males  and attributed primarily to the Y-associated autoimmune accelerator (*Yaa*) locus of the SB/Le male.
* NZB mice can develop a multitude of autoimmune phenotypes characterized by hypergammaglobulinemia with high levels of circulating autoantibodies, Coombs positive hemolytic anemia, and immune complex glomerulonephritis and a lymphoproliferative disorder involving B cells. NZW mice develop glomerulonephritis and autoantibodies; females are more likely than males to be affected. NZB/W mice, which are the F1 hybrid offspring of NZB females and NZW males, develop a life-limiting autoimmune condition that is more severe in females.
* NZM2410 mice develop autoimmune glomerulonephritis at an early age in both males and females.

Important Spontaneous Mutations: See Supplementary Table 2 for a comprehensive overview of well-known immune relevant mutations in mice that exhibit Mendelian inheritance. Examples described in the text are the characterization of TLR4, which was first recognized as the main sensor for lipopolysaccharides, based on studies conducted in C3H/HeJ mice, which are spontaneously TLR4 deficient. Spontaneously recessive mutations in the *scid, Lystbg, and Btkxid* genes have been important for studying orthologous conditions in humans and other animals. The *scid*and *nu* mutations have been particularly important in the context of xenotransplantation studies. Mice homozygous for either spontaneous recessive mutation of *Faslpr*or *Fasgld*develop lymphoproliferative and autoimmune diseases related to defects in the FAS-mediated apoptosis pathway. The *gld* mutation arose spontaneously in C3H/HeJ mice.

Interactions Among Mutations:See Table 3 for summary. Interbreeding to combine multiple mutations/hereditary disorders in one animal has proved useful for xenotransplantation studies and in the understanding the epistatic interactions among immune relevant genes.

* *Scid-*beige mice homozygous for both *Pkrdcscid* and *Lystbg*alleles combine the impaired B and T cell development of the *Pkrdcscid* mouse with the defective NK cell function of the *Lystbg*mouse. The result is mice that are severely immunodeficient and lack the leakiness associated with the *Pkrdcscid* animals.
* The *Foxn1nu* mutation causes congenital T cell deficiency. When combined with the *Faslpr*mutation, double mutants do not develop the autoimmune and lymphoproliferative disorders associated with the *Faslpr*mutation.
* Other models are discussed in a companion article in this issue of ILAR.

Induced Immunodeficiencies: Experimental interventions such as exposure to irradiation, chemicals, microbial organisms, biologic materials and surgical manipulation can result in intentional or unintentional modulation of the mouse immune system. Table 4 in the text provides examples from the major categories.

* Ionizing radiation has historically been used to suppress the immune system in mice and other animals, and remains an important part of the regimen to induce immunosuppression in animal models of allo- or xenotransplants and stem cell transplant for the generation of mice with humanized immune systems. *Pkrdcscid* mice have impaired ability to repair radiation-induced DNA double strand breaks and so are particularly radiosensitive. BALB/c mice strains are highly sensitive to radiation, while C57BL/6, A/J, and C3H/HeMs are highly resistant. High doses of gamma irradiation are known to cause immunosuppression, with mouse B cells shown to be more sensitive than T cells. Repeated low-dose gamma irradiation can modulate the immune system and lead to a robust Th2 response, which may mitigate autoimmune conditions that are Th1 response-dependent. Mice with defective adaptive immunity, such as nude and *scid* mice, can generally control most opportunistic infections. However once they have been irradiated and lose their innate immunity, overwhelming infection becomes an important cause of mortality in these mice. UV radiation may be used to study the effects on local skin immunity in research on photocarcinogenesis or inflammatory skin conditions.
* Chemicals that have been used to experimentally induce immunosuppression include alkylating agents, metals, aromatic hydrocarbons, and antimicrobial agents. Cyclophosphamide (CYP) induces neutropenia and mice treated with CYP have impaired granulocyte production and/or leukocytic function and are prone to develop systemic disease when infected with environmental opportunistic pathogens. CYP immunosuppression results from induction of apoptosis in activated B and T cells and NK cells. At low doses CYP may enhance immune responses to tumor antigens, in part due to suppression of regulatory T cells (Tregs).  Busulfan can enhance engraftment of xenotransplanted hematopoietic stem cells. 5-fluorouracil (5FU) promotes activation of tumor-specific CD8+ T cells. Tacrolimus and cyclosporine A are calcineurin inhibitors (CNI), which directly inhibit Tregs function. Glucocorticoids have anti-inflammatory and immunosuppressive effects. Estrogens and androgens can suppress both adaptive and innate immunity. Nanoparticles used as drug delivery mechanism or biomedical imaging tool, have unique characteristics that can influence their interaction with the host’s immune system and the overall immunotoxicologic profile.
* Biologics, such as antibody-mediated depletion of cell lineage-specific immune effector cells, have been used to target specific functions of the mouse immune system. Anti-thymocyte globulin specifically depletes T cells in NOD mice. Glucans, CpG oligodeoxynucleotides and bacterial endotoxins have also been used.
* Surgical interventions such as thymectomy and splenectomy can be used to alter immunity. Thymectomy effects T cell development, however mice have a high frequency of ectopic functional thymic tissue which can be a confounding source of T cells. NOD and BALB/c mice reportedly have ectopic thymic tissue more commonly than C57BL/6 mice. Splenectomy has been used to study the role of the spleen in infectious diseases, tumor growth, and peripheral antigen tolerance.

Induced Autoimmune and Hyperimmune Conditions: Autoimmune diseases arise from poor control of self-reactive lymphocytes and cytokine production or disrupted Tregs and effector T cell balance. There may be genetic predisposition for these conditions, but manifestation of disease usually depends on additional immune triggers, such as infection, tissue damage or dysbiosis. In mice, strain-related variations in innate and adaptive immunity affect penetrance, onset, and severity of disease.

* Susceptibility to rheumatoid arthritis (RA)-like conditions in mice depends on multiple alleles and QTL. These conditions can be induced using type II collagen-induced arthritis (CIA) or proteoglycan-(aggrecan)-induced arthritis (PGIA). Mouse strains expressing H-2q and H-2r haplotypes are most susceptible to CIA. BALB/c (H-2d) mice are more susceptible to PGIA than CIA, whereas DBA/1 (H-2q) are sensitive to CIA but insensitive to PGIA. DBA/2 (H-2d) mice are resistant to arthritis induced by either method.
* Multiple sclerosis (MS) does not exist as a spontaneous mouse model, but aspects of the disease can be recapitulated by EAE induction. SJL/J, C3H/HeJ, C57BL/6, and DBA1 strains are susceptible to EAE induction, and SJL/J mice in particular are used to model features of relapsing-remitting MS. GEM models with specific myelin-targeting immune cells have also been used, as have mouse hepatitis virus (MHV)-induced demyelination models.

Other Immunomodulators and Unintended Experimental Consequences**:** See summary in Table 5 for examples of common environmental factors that can have an effect on the mouse immune system.

* Many factors in the micro- and macroenvironment can be stressors for mice, which activate the hypothalamic-pituitary-adrenal axis and increase circulating glucocorticoids. Corticosterone is the primary stress-induced glucocorticoid in mice. Common factors include caging, housing density, enrichment, temperature, humidity, illumination, noise and vibration, bedding, diet, water, and husbandry and biosecurity. Nesting material provided as enrichment generally has a positive effect on several immune parameters and helps reduce the stress level for mice. As current temperature recommendations for mouse housing are below the mouse thermoneutral zone of 30-32°C, most mice in a research setting experience “mild” cold stress, which can affect immune responses and tumor growth. Exposure to light and circadian rhythm alterations are recognized to effect the immune system in many species. Dysregulation of circadian rhythm in mice induces a generalized proinflammatory state and exacerbates diet-induced systemic insulin resistance and glucose intolerance. Phytoestrogens present in commercial research diets can cause endocrine disruption and can influence rodent reproduction, immunity, cardiovascular system, and neoplastic conditions. The importance of reporting environmental and husbandry conditions, along with specifics of experimental or therapeutic interventions in scientific publications cannot be overstated.
* Systemic and mucosal immunity in mice are influenced by intestinal flora (microbiota). Autochthonous (commensal and symbiotic) microbiota such as segmented filamentous bacteria (SFB) are important in inducing Th17 responses in mice, and have been shown to influence neuroinflammation in EAE models as well as diabetes susceptibility in NOD mice. Flora with more species of *Bacteroides* and *Parabacteroides* may mitigate DSS-induced colitis. There can be strain-associated and/or vendor-dependent differences in gut microbiota in mice that can confound or increase variability in research results.
* Allochthonous (noncommensal) organisms can cause morbidity, mortality and other adverse effects on research. Viruses such as murine parvoviruses, herpesviruses, and retroviruses have selective tropism for immune cells. Murine parvovirus is lymphocytotropic and alters CD4+ and CD8+ T cell responses during acute infection. Mouse thymic virus causes thymic necrosis and transient immunosuppression in infected neonatal mice. Retroviruses (both exogenous and active endogenous) are lymphocytotropic and have roles in lymphoproliferative disorders as well as mammary carcinogenesis, sarcoma development, and lymphoma development. Murine and human viruses and bacteria can exist in biological materials, including transplantable tumors, cell lines, serum, and embryos.
* Genetic engineering strategies can have unintended or unexpected effects on the mouse immune system. Cre/loxP- based recombination technology can result in a strong antiviral response due to the endonuclease activity of Cre recombinase. Tamoxifen-inducible Cre/loxP system can lead to a shift from Th1 to Th2-mediated immune response due to the estrogen effects of tamoxifen. Other strategies such as tetracycline-controlled transcriptional activation, expression of fluorescent or enzymatic reporters, and the CRISPR-Cas9 system can have effects influencing the immune system and producing more variables in research studies.

Future Directions: The use of humanized mice or other animals used to study human-derived immune elements in nonhuman surrogates are one option being explored. Genetic approaches include factorial design study and Collaborative Cross (CC)-derived RI strains and Diversity Outbred (DO) mice. There has been recent interest in the use of genetically and microbially “wild” mice as a more human relevant strategy.

QUESTIONS

1. Under similar conditions, leakiness is greater for scid mice on which background strain?
2. NOD
3. C3H/HeJ
4. BALB/c
5. MRL/MpJ
6. Which inbred strain spontaneously develops lupus-like conditions?
   1. NZB
   2. NZW
   3. MRL/MpJ
   4. BSXB/MpJ
   5. All of the above
7. Which mouse strain is highly sensitive to ionizing radiation?
   1. A/J
   2. DBA1
   3. C57BL/6
   4. BALB/c
8. Mouse strains expressing which haplotypes are more sensitive to induction of collagen-induced arthritis (CIA)?
   1. H-2q and H-2r
   2. H-3q and H-2s
   3. H-3q and H-3r
   4. H-2i and H-2v
9. NZB/W mice are the F1 hybrid offspring of which of the following crosses
   1. NZB female and NZW male
   2. NZB male and NZW female
   3. NZW male and NZW female
   4. NZB female and NZW/B male
10. Mice on the C57BL/6 background strain are:
    1. Unlikely to display the leaky phenotype associated with the *scid*mutation
    2. Very likely to develop severe immune complex pathologies with the *Faslpr*mutation
    3. Have more ectopic thymic tissue than other strains
    4. Are susceptible to EAE induction
11. Thymectomy affects
    1. B cell development
    2. T cell development
    3. NK cell development
    4. Cytokine production
12. Which of the following environmental factors is associated with positive effects on immune parameters and reducing stress levels in mice?
    1. Mild cold stress
    2. Circadian rhythm dysregulation
    3. Commercial diets high in phytoestrogens
    4. Provision of nesting materials

ANSWERS

1. c

2. e

3. d

4. a

5. a

6. d

7. b

8. d

**Overgaard et al.** [**Of Mice, Dogs, Pigs, and Men: Choosing the Appropriate Model for Immuno-Oncology Research**](https://academic.oup.com/ilarjournal/article/59/3/247/5196515)**, pp. 247-262**

Domain 3: Research, K3 animal models

Primary Species: Mouse (*Mus musculus),* Dog (*Canis familiaris),* Pig (*Sus scrofa)*

SUMMARY: One universal animal model for preclinical testing or studying the complex pathways of tumor-immune cell interactions does not seem realistic when studying cancer because cancer is not a single disease, and different tumor types require specific treatment strategies.  Mice have been the most commonly used animal model for immunological research.  GEM mouse models are very useful for studying the effect of specific mutations on tumor progression in an immunocompetent host, but they often fail in mimicking the complexity of human tumors.  Despite manipulation with sophisticated genetic techniques, xenograft mouse models often have limitations in imitating the immune-mediated pressures and humanized mice are devoid of key organized immune microenvironments critical to initiating robust immune responses.  Canine and porcine models of human cancer are uniquely suited to serve as excellent comparative tumor models considering their comparable body size and metabolic physiology to humans, and their well-annotated genomes.  The canine immune system demonstrates a close homology to the human counterpart and many of the same immune markers have been validated in the canine species.  Strategies have been developed for stimulating the innate and adaptive, both passive and active, immune system components in canine cancer models.  Pigs and humans have a high degree of homology in anatomy, physiology, size, cell biology, key metabolizing enzymes, genetics, and epigenetics.  Pigs are more closely related to humans at the immunome level than mice.  Porcine skin is very similar to the morphology and functional characteristics of human skin.

Cancer immunoediting describes a complex interplay in which the immune system protects against cancer, as well as inducing tumor-sculpting mechanisms, which lead to reduced immunogenicity of tumor cell variants.  The concept of cancer immunoediting is composed of 3 phases: elimination, equilibrium, and escape.  The spontaneous canine tumor models provide a unique platform for evaluating therapies aimed at the escape phase of cancer while genetically engineered swine allow for elucidation of tumor-immune cell interactions especially during the phases of elimination and equilibrium.

QUESTIONS

1.  T/F: Cancer is the leading cause of death worldwide.

2.  Define the term immunosurveillance.

3.  Match the correct definition with the term, as it relates to cancer immunoediting.

a.  Elimination

b.  Equilibrium

c.  Escape

i.   Supression of antitumor immunity and/or lack of recognition.

ii.  Active immunosurveillance

iii.   Dynamic equilibrium between the tumor and the immune system.

4.  T/F: Humanized mice are either genetically engineered to carry human genes or developed through engraftment of human immune cells into an immunodeficient host.

5.  Which of the following cancer types is mimicked by both a spontaneous canine model and a genetically engineered porcine model?

a.   Transitional cell carcinoma

b.   Hepatocellular carcinoma

c.  Lymphoma

d.   Osteosarcoma

e. All of the above

ANSWERS

1.  True. It has surpassed cardiovascular disease.

2.  Immunosurveillance has traditionally been used to describe how the immune system can protect the host from tumor development.

3.  a-ii, b-iii, c-i

4.  True

5.   Both c & d; canine a; porcine b

**Graves et al.** [**Animal Models for Preclinical Development of Allogeneic Hematopoietic Cell Transplantation**](https://academic.oup.com/ilarjournal/article/59/3/263/5052232)**, pp. 263-275**

Domain 3: Research; K3. animal models (spontaneous and induced) including normative biology relevant to the research (e.g., background lesions of common strains)

Primary Species: Mouse (*Mus musculus*) and Dog (*Canis lupus familaris*)

SUMMARY: Since1950s Hematopoietic stem cell transplantation (HSCT) remains an essential therapeutic modality for treatment of a diverse array of diseases, including a multitude of hematological malignancies, autoimmune disorders and inherited genetic hematological disorders. Although great advances have been made in understanding and application of this therapy, significant complications still exist, warranting further investigation. Of critical importance disease relapse and graft-versus-host disease (GVHD), in both acute and chronic forms, remains a major complication of hematopoietic stem cell transplantation, responsible for both the development of chronic illness and morbidity, as well as mortality. Use of an appropriate preclinical animal model may provide significant insight into the mechanistic pathways leading to the development and progression of graft-versus-host disease, as well as transplantation in general. However, existing preclinical modeling systems exhibit significant limitations, and development of models that recapitulate the complex and comprehensive clinical scenario and provide a tool by which therapeutic intervention may be developed and assessed is of utmost importance. In the present review, the authors discussed various animal models of allo-HSCT and their significant contribution in the field of HSCT. Authors also compared HSCT manifestations in animal models to that of humans and summarized the preclinical models currently in use, as well as their advantages and limitations.

QUESTIONS

#### 1.   Experiments and observations in which of the following animal species encouraged further trials of marrow grafting between human leukocyte antigen (HLA)-matched human siblings?

a. Mice

b. Dogs

c. Pigs

d. Non-human Primates

2. Which of the following is the most common cause of death following allogeneic stem cell transplantation?

a. Infections

b. Graft-versus-host disease

c. Relapse of the underlying condition

d. Idiopathic pneumonitis

e. Drug-induced renal failure

3.   Which of the following will INCREASE the likelihood of developing graft-versus-host disease (GVHD) in allogeneic transplantation?

a. Cyclosporine

b. Methotrexate

c. Donor lymphocyte infusions (DLI)

d. T-cell depletion of the graft

e. Mycophenolate

4. Which of the following statements is INCORRECT regarding autologous transplantation?

a. The majority of transplants are performed to treat lymphoma and myeloma

b. Relapse is the most common cause of death following autologous transplants

c. Stem cells are collected from the patient after a period of priming with G-CSF

d. The graft-versus-leukemia effect is a major component of the therapeutic effect

5.   Murine models of chronic GVHD do not adequately recapitulate human disease because?

a. Fibrosis in human chronic GVHD can be systemic or pleiotropic and in mouse, it is tissue specific

b. The repertoire of autoantibodies generated  are more diverse in humans

c. Splenomegaly and lymphadenopathy do not occur in human

d. Nephritis in human GVHD is rare but common in the mouse model

e. All of the above

6.   Acute and chronic GVHD are induced by donor T cells. Which tissue is not considered a primary target?

a. Heart

b. Skin

c. Lung

d. Liver

e. Intestinal Tract

7.  Which of the following drugs are used to prevent or treat acute GVHD? ( Chose More than one drug)

a. Cyclosporine (CSP)

b. Methotrexate

c. Tacrolimus

d. Mycophenolate mofetil (MMF)

e. All of the above

8.   True or False: The most common MHC- mismatched model of acute GVHD is a transplant from C57/Bl6 (H2b) donors to Balb/c (H2d) recipients?

9.  True or False: Four types of radioisotopes used for Antibody-radionuclide conjugates are Iodine-131, yttrium -90, astatine-211 and bismuth-213?

ANSWERS

1.  b

2. c

3.  c

4.   d

5.  e

6.  a

7.   e

8.  True

9.   True  ( β emitters: Iodine-131 and yttrium -90; α emitters: astatine-211 and bismuth-213)

**Migliorini et al.** [**Keeping the Engine Running: The Relevance and Predictive Value of Preclinical Models for CAR-T Cell Development**](https://academic.oup.com/ilarjournal/article/59/3/276/5490287)**, pp. 276-285**

Domain 3

SUMMARY: This paper is all about the history, current status, and future of Chimeric Antigen Receptor (CAR) T Cell therapeutic models.  It covers the advances that have happened and the challenges that have yet to be overcome.  CAR T Cells are created by obtaining cells via leukapheresis from the patient, activating them, and expanding them.  They are then transduced with retroviral virions to express the CAR that is specific for the cancer the patient has.  The cells are then expanded in numbers and reinfused into the patient.  The primary advantage of this approach is that the CAR T Cells are the same MHC as the patient and therefore there is less rejection.

In 2017 the FDA granted approval for B cell malignancy therapy using CAR T Cells.  This was accomplished following dramatic improvements in patient outcomes, especially with refractory cases.  The CAR T Cells were created to target a B cell specific receptor (CD19) which did cause the expected B Cell aplasia (expected based on mouse models), but it also caused other problems which were not expected (Cytokine Release Syndrome, Macrophage Activation Syndrome, and neurotoxicities).

Animal models (primarily mouse models) have been instrumental in the preclinical investigation and development of CAR-T cell therapies.  The most commonly used mouse models are:

* + - Immunocompetent (Syngeneic/Transgenic) – this is useful because it uses a mouse with a full immune system, however you much use CAR T Cells of murine origin as human cells would not work.  This ultimately limits the translatability of the model.  One way around this would be to engineer a mouse cell to express human antigens.  Spontaneously developing tumors are useful as they can serve as a target for mouse CAR T Cells.
    - Immunodeficient (Xenograft/PDX) Mice – This is the most commonly used model.  Mice implanted with human tumor cells and treated with human CAR T Cells.  Translatability is potentially higher, but other elements of the immune system are not present.
    - Humanized Mice - Immunodeficient animal models are limited in their ability to interrogate interactions between the innate and adaptive arms of the immune system. In this regard, humanized mouse models, reconstituted with human CD34+- cells in their simplest format, can overcome these obstacles.
    - Dog Models - Canine cancer patients provide the opportunity to study acute toxicities of CAR-T cells in the spontaneous rumor setting and in the presence of an intact immune system.  This system is not without its limitations.  The process of engineering and expanding canine CAR T Cells is not as advanced as it is for humans and mice.
    - NHP Models – Obviously the translatability would be high in a primate model, but this is usually done at a later stage in development of a therapy.   The major drawback to using NHPs (other than cost and ethics) is that induced tumor models do not really exist.  However, they can be used as a model of CAR T Cell mediated neurotoxicity and Cytokine Release Syndrome.  NHP models would also be useful for Persistence studies and off target effect studies.

QUESTIONS

1.   Which of the following would NOT be considered a part of the Cytokine Release Syndrome complex?

a.   Hypertension

b.  High Fever

c.   Vascular Leakage

d.  Liver Failure

2.  Which of the following would be considered a good marker for B Cells?

a. CD4

b.  CD8

c.   CD19

d.   CD32

3.  The simplest form of humanized mouse model involved reconstituting the mouse with \_\_\_\_\_\_+ cells.

a.   CD8

b.  CD16

c.   CD34

d.   IL2

e.   IL8

ANSWERS

1.   a

2.  c

3.  c

**Platt et al.** [**Xenotransplantation: Progress Along Paths Uncertain from Models to Application**](https://academic.oup.com/ilarjournal/article/59/3/286/5239653)**, pp. 286-308**

Domain  3: Research

SUMMARY:In this publication the authors were aiming at summarizing the rationale for pursuing xenotransplantation of organs/cells into humans, the obstacles to success, and recent advances in overcoming those obstacles. They deliberately avoid consideration of fundamental advances that are not pertinent to practical applications.

Immunity is still the main obstacle to successful transplantation between different individuals, and the clinical practice of transplantation today is predicated on the continuous provision of immunosuppressive agents (and on availability of antimicrobial agents to address toxicities imposed by immunosuppression).

However, immunity t is not the only barrier. Despite the availability of powerful and highly effective regimens of immunosuppression, up to one-half of all allografts ultimately fail over time. Which grafts are likely, to fail and why some fail and some persist are subjects of intense research.

The limited supply of human organs and tissues for transplantation remains the preeminent rationale for developing xenotransplantation as an alternative to allotransplantation.

Organ failure significantly affects the outcome of clinical transplantation, increasing the risk of infection, early graft failure, and other complications. Unfortunately, few if any of the pre-clinical (i.e., large animal) models used to investigate transplantation faithfully represent this impact. This lack of models delays the development of therapies, in particular when the transplant is to be performed on already ill patients, in which the modelling of the disease itself is critical.

The CRISPR-Cas9 system is now being used to modify pigs, to directly target the synthesis of antigenic targets.

Another approach is reverse xenotransplantation, in which animals are used to generate and expand human cells. As an example, this approach could be used to generate hepatocytes, islets or hematopoietic cells. Reverse xenotransplantation in pigs could offer several advantages, among them the large body of research regarding rejection of the transplants. However, the body of literature regarding reverse xenotransplantation in pigs is limited.

An absolute requirement for engraftment of human cells in other individuals of the same or disparate species is suppression or elimination of adaptive immunity. The authors and other research groups have begun to use genetic engineering to produce immunodeficient pigs. They have achieved targeted disruption of RAG2, RAG1 and IL2RG. These pigs accept allogenic stem cells and reconstitute the immune system. However, when xenogeneic cells are used, they see that hurdles beyond innate and adaptative immunity limit xenogeneic engraftment.

NHP have been excluded as source of clinical xenografts due to infectious risks and scarcity. Additionally, although the physiology of NHP resembles that of humans, their smaller size limits the impact the organs would have as xenografts in mature humans.

Pigs do not present these problems. They also can be genetically engineered and have sizable litters. Infectious diseases transmission is well understood due to their long coexistence with humans.

The intersection of immunity, physiologic incompatibility, and infection underscore the importance of taking account of the limits of simplified experimental systems, such as cell cultures and small animals, in predicting the impact of the various barriers as they would be manifest in swine-to-human transplants.

Immunity has been considered the most daunting barrier. Recent reports have achieved longer than one year survival of heterotopic cardiac and kidney xenografts.

Xenotransplantation recruits every facet of innate immunity. The most dramatic example is the early pathogenic impact of activation of the complement. A possibility would be to express small amounts of human complement regulators in transgenic pigs.

The natural antibodies of greatest interest in xenotransplantation are natural antibodies specific for Galα1-3Gal. Mammals lacking Galα1-3Gal produce natural antibodies specific for that saccharide. This has led to the production of Gal KO pigs.

Cellular elements can recognize foreign or injured autologous cell surfaces and products released from cells and initiate the ensuing reactions. They can also be recruited to inflammatory reactions begun by other recognitive systems. Cellular elements can also play a tangential role in pathogenesis (i.e. injury due to complement). However, is not entirely clear whether interactions between macrophages, NK and other cells and xenografts initiate or cause the greater inflammatory reactions associated with xenotransplantation.

Elicit antibody responses might also limit the success of xenotransplantation.

The biochemical incompatibilities can range from little to severe depending on the organ and the species involved. Lungs, kidneys, hearts and transplants can temporarily support the life of NHP. In contrast, orthotopic porcine liver xenografts can engender life-threatening complications (i.e. thrombocytopenia). Pigs expressing multiple transgenes could address these issues.

The potential usefulness of xenotransplantation in clinical settings remains a matter of speculation. NHP do not model the well the diseases addressed by transplantation. Only clinical trials can be expected to test the efficacy for some potential applications of xenotransplantation. Two exceptions might be transplantation of islets for diabetes and hepatocytes for acute liver failure.

QUESTIONS

1. The barriers for xenotransplantation are:
   1. Immune response
   2. Physiological and biochemical incompatibility
   3. Potential for transmission of infectious diseases
   4. All of the above
2. Innate immunity in heterotopic transplants is due to:
   1. Response to ischemia-reperfusion
   2. Recognition of the graft as foreign
   3. Endothelium incompatibility
   4. a+b
3. Which of the following animals do not produce Galα1-3Gal?
   1. New world monkeys
   2. Pigs
   3. Humans
   4. Mice
4. The factors determining the impact of innate and adaptative immunity in xenografts are:
   1. The source of blood vessels in the graft
   2. Intrinsic and induced resistance of the graft to inflammatory injury
   3. Nature and kinetics of immunity
   4. All of the above

ANSWERS

1. d

2. d

3. c

4. d

**Tandrup Schmidt et al.** [**Rational Design and In Vivo Characterization of Vaccine Adjuvants**](https://academic.oup.com/ilarjournal/article/59/3/309/5281128)**, pp. 309-322**

Domain 3

SUMMARY: Most vaccines are based on whole, inactivated, or attenuated pathogens, some of which have been adjuvanted with aluminum salts. Aluminum-based adjuvants have been extensively used and for a while were the only adjuvants approved for human use. Traditional vaccines typically induce a strong neutralizing antibody response and the target pathogens do not change their surface structure over time (e.g.- measles, mumps, and diphtheria). In order to create effective vaccines for more challenging and complex pathogens (e.g.- HIV, pandemic flu, tuberculosis, and cancers), novel vaccine formulations are necessary which will induce associated humoral and cell-mediated or cytotoxic T-cell responses as necessary.  These novel vaccine adjuvants are increasingly designed to induce specific immune responses. Rational design of vaccine adjuvants can be applied by taking into account antigen type, target cell subsets, and immunostimulators, but requires knowledge of the following aspects:1) the required immune response to prevent infection/disease from a given pathogen, 2) the specific innate immune cells necessary to induce said immune response, 3) the location of these innate cell populations, and 4) which pattern recognition receptors (PRR) the cells express.

The evaluation of the biodistribution and cellular association of adjuvanted vaccines is essential knowledge for the induction of specific immune responses. Knowing immune cell responses in response to different routes of injection and draining lymph nodes from a given injection site provide valuable information. Mapping the biodistribution pattern of a vaccine provides info about which compartments are affected, allowing one to assess if the correct organ and cell types are targeted to induce the beneficial immune response. Confocal microscopy, flow cytometry, and immunofluorescent staining with microscopy are all useful methods for evaluating the components of the immune response. This characterization of the association of the vaccine adjuvant and antigen with specific innate cell subsets at the injection site and lymphoid organs can be used to identify cell-mediated transport to the injection site and provide insights into the antigen presenting cell (APC) subsets priming the immune response. Flow cytometry combined with high-throughput imaging of immunofluorescent staining represents a promising new technique for investigating innate immune cell targeting, uptake, and subcellular location. Injection of radiolabeled vaccine particles can allow for a quantitative evaluation of vaccine biodistribution. Characterization of immunostimulators that activate specific immune cell subsets is important and can often be evaluated in vitro. However, due to the complexities inherent in the immune system to induce and maintain antigen-specific immune responses, animal models are preferred for functional evaluation of vaccine candidates.

Antibody response is the best-established correlate of protection against many diseases. The antibody responses can be measured as antibody titer by enzyme-linked immunosorbent assay (ELISA) or by disease-specific approaches such as virus or bacterial neutralization. Vaccination elicits B-cell activation in draining lymph nodes, followed by formation of germinal centers, in which affinity maturation and class switching occurs. Adjuvants may affect the magnitude of germinal center responses, which can be measured by flow cytometry or immunofluorescent staining and confocal microscopy. Germinal center B-cells ultimately become one of two cell types: memory B-cells or plasma cells. The former  are generated primarily by early germinal centers while the latter require more progressed germinal centers. Memory B-cells live in the circulation and can be quantified and followed using flow cytometry and FACS. Standard evaluation of vaccine-induced antibody responses includes determination of antigen-specific serum IgG levels. Evaluation of antigen-specific CD4 and CD8 T-cells induced by novel vaccine formulations is also an important measure of vaccine efficacy. CD8 T-cell responses induced by immunization can be evaluated for the level of cytokine producing cells in response to the stimulus. Induced CD4 T-cells are critical for functional immune responses: Th1 responses induce proinflammatory responses and help induction and sustaining of CD8 T-cell responses, while Th2 responses help promote antibody class switching and Th17 response is important for establishing mucosal immune responses. Stimulation of single-cell suspensions from target organs with the subunit antigen and minimal CD8 epitope peptides is a well-established method for evaluating antigen-specific CD4 and CD8 cells, respectively. Because flow cytometry has limitations to the number of antibodies that can be analyzed at one time, an alternative method for evaluation of T-cell populations is cytometry by time-of-flight (CyTOF), where antibodies are conjugated to heavy metal isotopes. However, the data acquisition rate is lower compared to flow cytometry. The CyTOF method requires low sample volumes and may have potential for assessing changes in immune cell type and functionality after immunization with different subunit vaccines. ELISPOT can also be used to assess cytokine levels in response to antigen stimulation, and can be useful in animal models where flow cytometry antibodies are scarce. Epitope-mapping, where splenocytes from immunized mice are stimulated with individual peptides spanning the entire protein, can be used to identify CD4 and CD8 epitopes in novel protein or peptide-based antigens. The proliferation of antigen-specific CD4 and CD8 T-cells upon therapeutic vaccination can be used as a measure of how well the cells respond to vaccination. There are different assays which can measure cell-mediated cytotoxicity, which can help assess the efficacy of vaccines. The 51Cr release assay is the gold standard.

The transfer of experimental vaccines and adjuvants from preclinical work to clinical trials remains a hurdle in vaccine development. Animal models are essential but the choice of animal model depends on many factors and is worth careful consideration. The majority of in vivo vaccine efficacy studies are performed using inbred mice. While the innate immune system is conserved across multicellular organisms, the adaptive immune system is unique to vertebrates. Some of the differences between the humoral immune response in animal models and humans highlighted in this article included: 1) differences in the immune cell compositions and functions; 2) differences in the Ig isotypes and particularly the production of IgG1; 3) differences in mucosal IgA response; and 4) FcR expression variability. With regards to comparing cell mediated immunity, the biggest challenges is that in animal models the responses are measured in lymphoid organs while in humans they are measured in blood. Studies have documented good correlation between mice and men in regard to Th1 and Th2 responses, however information is still needed on correlates between induction of other T-cell subsets, such as Th17, Treg, and Tfh. Th17 CD4 cells are crucial for mucosal immunity and protection against pathogen entry. They are also able to produce the cytokine IL-17. Treg cells function to maintain immunological homeostasis and prevent excessive inflammation, and thus may interfere with vaccine-induced immunity. The biggest challenge to more fully evaluating these cell subset responses is the difficulty in obtaining the relevant tissues from human subjects.

QUESTIONS

1. What is currently the most common and most extensively used vaccine adjuvant?
   1. Liposome adjuvants
   2. Sodium-based adjuvants
   3. Aluminum-based adjuvants
   4. Gold nanoparticle adjuvants
2. Which of the following is involved in the rational design of vaccine adjuvants?
   1. Targeting of the appropriate cell populations with the right co-stimuli
   2. Knowledge of the immune response required to prevent disease
   3. Presenting sufficient amounts of the right antigen in the right conformation
   4. All of the above
3. T/F: The route of adjuvant injection may affect the immune response.
4. What is the best-established correlate of protection against many (but not all) diseases?
   1. Antibody response
   2. Viral titers
   3. Quantification of antigen presenting cells
   4. Effector cytokine response
5. Germinal center B-cells ultimately become either \_\_\_\_\_\_\_\_\_ or \_\_\_\_\_\_\_\_\_\_\_\_.
   1. Memory cells ; macrophages
   2. Memory cells; plasma cells
   3. Plasma cells; macrophages
   4. Plasma cells; Tfh cells
6. The standard evaluation of vaccine-induced antibody response includes determination of antigen-specific serum \_\_\_ levels.
   1. IgA
   2. IgE
   3. IgG
   4. IgM
7. Which CD4 T-cell subset seems to be important in establishing mucosal immune responses?
   1. Th1
   2. Th2
   3. Th17
   4. Th22
8. Which CD4 T-cell subset promotes antibody class switching?
   1. Th1
   2. Th2
   3. Th17
   4. Th22
9. T/F: the innate immune system is unique to vertebrate mammals.

ANSWERS

1. c
2. d
3. T
4. a
5. b
6. c
7. c
8. b
9. F

**Starbaek et al.** [**Animal Models for Influenza A Virus Infection Incorporating the Involvement of Innate Host Defenses: Enhanced Translational Value of the Porcine Model**](https://academic.oup.com/ilarjournal/article/59/3/323/5196521)**, pp. 323-337**

Domain 3: Research

Primary Species: Pig (Sus scrofa)

SUMMARY: Influenza is a respiratory disease caused by influenza A virus (IAV) with influence in public health. Although for most people is a transient mild disease, there are human risk groups, like elderly, morbidly obese o those persons with inflammatory conditions, in which it can be a fatal disease. In this article authors reviewed and discussed different small and large animal models for study human IAV infection. They focused in pigs, because pigs present many similarities with humans: are natural hosts for same IAV subtypes, its clinical disease mirror humans’ symptoms, lung anatomy and physiology are similar as well as immune response to infection. In addition, pig models with underlying inflammatory diseases can have severe IAV disease and impaired vaccine responses as humans do.

When comparing different animal models, those with pigs and ferrets show high molecular similarities (protein involved in viral recognition, interferon responses and interferon-stimulated genes responses) with human than murine models.

Authors concluded that pigs are promising translational models to humans related with IAV infection, pathogenesis and immunity.

QUESTIONS

1. T/F: Influenza A Virus (IAV) causes high morbidity and mortality in humans, mainly in risk groups as elderly or infants

2. T/F: High risk human groups tend to have short and low invasive forms of IAV infections

3. T/F: Influenza A virus are non-enveloped double-stranded viruses

4. What is the reference to classify Influenza A virus in different subtypes?

5. What is the difference between antigenic drift and antigenic shift in a virus?

6. New virus subtypes are generated by combination of antigenic \_\_\_\_\_\_\_\_\_ and\_\_\_\_\_\_\_

7. What is the most used animal model in human IAV research?

a. Rodents

b. Ferret

c. Nonhuman primates

d. Pig

8. T/F: Mouse model provided extensive knowledge as translational model for IAV research

9. T/F: Mouse show different clinical signs than humans because they are not naturally infected by IAV.

10. What adaptations need to be done in IAV that allow it to replicate in mice?

a. Mutation in receptor-binding site of viral hemagglutinin protein

b. Mutation in receptor-binding site of viral neuraminidase protein

c. Loss of glycosylation sites

d. All of above

11. What can be the most important change when mutations in IAV genome are introduced?

12. What two animal species can be used as animal models for IAV studies? Why?

13. T/F: Pig models reflect human anatomy and IAV pathogenesis infection more faithfully than mouse models

14. T/F: Mouse models reflect human anatomy and IAV pathogenesis infection more faithfully than pig models

15. T/F: Pig breeds showed different clinical manifestations when infected with human IAV.

16. T/F:  As ferrets, pigs infected with human IAV are inefficient transmitting it to other healthy pigs

17. For human IAV study, when we will need more animals, using pig models or mouse models?

18. T/F: In contrast to pigs, sensitivity of mice to bacterial lipolysacharide is like humans

19. T/F: In contrast to mice, sensitivity of pigs to bacterial lipolysacharide is like humans

20. What signs are present in mice with human IAV infection?

a. Fever

b. Nasal secretion

c. Coughing

d. None of above

21. T/F: In contrast to pigs and ferrets, mice do not possess tonsils

22. T/F: As pigs and ferrets, mice possess tonsils

23. What of the next apply to pigs?

a. Thick pulmonary pleura

b. Possesses tonsils

c. Pulmonary connective tissue is interlobular

d. All of above

24. T/F: In contrast to pigs, mice and ferrets have little pulmonary connective tissue

25. In what specie pulmonary intravascular macrophages are constitutive phagocytic cells?

a. Pig

b. Human

c. Ferret

d. Mouse

26. Define respiratory mucus layer

27. What of the next have not mucus secreting goblet cells in the upper respiratory tract?

a. Ferret

b. Pigs

c. Mice

d. Humans

28. T/F: Pigs and Ferrets have a large proportion of ciliated respiratory epithelial cells in tracheal surface

29. Why in mice infection with IAV is located in lower respiratory tract instead of upper respiratory tract?

30. T/F: Amino acid sequences of antiviral proteins associated with human IAV infection in pigs, ferrets and mice are like to humans

31. T/F: Pig model can provide a way to study systemic transcriptional response to IAV infections

32. T/F: Pig model can provide a way to study local pulmonary response to IAV infections

33. What are host-encoded microRNA?

34. Can microRNA have translational importance in pig models of human IAV?

35. What means face validity of animal model?

36. What means target validity of animal model?

37. What means predictive validity of animal model?

38. Related to human IAV studies, what type of animal models have to be prioritized?

39. T/F: animal models with high face validity have also high target validity

40. T/F: Pig models have higher face validity than mouse models for human IAV infections

41. Which has the central role in human IAV infections?

a. Characteristic of respiratory tract

b. Innate immune mechanisms

c. Adaptative immune mechanisms

d. Cellular immunity

42. T/F: In contrast to pig models, mouse models of human IAV infection need the use of adapted IAV strain

43. T/F: Pigs and ferrets show high sequence similarity to humans in a wide range of antiviral proteins rather than mice

44. Among others, what made pigs a suitable model for study human IAV infections?

ANSWERS

1. T

2. F

3. F

4. Antigenic surface glycoprotein hemagglutinin and neuraminidase

5. Antigenic shift is when new virus subtypes are generated by new combinations of gene segments. Antigenic drift is due to high rate mutation of RNA genome that lead to amino acid changes in important viral epitopes

6. Shift, drift

7. a

8. F

9. T

10. d

11. Mutations introduced in IAV genome can induce important changes in phenotypic traits important for pathogenesis of IAV in humans

12. They can be infected with human IAV and show clinical signs resembling those in humans

13. T

14. F

15. F

16. F

17. Pigs models, because they are outbred and have large inter-individual biological variation

18. F

19. T

20. d

21. T

22. F

23. d

24. T

25. a

26. Is a complex viscous fluid secreted from globet cells and submucosal glands of the airway conducts

27. c

28. T

29. Because they have not globet cells and have lower levels of ciliated epithelial cells in upper respiratory tract

30. F

31. F

32. T

33. Endogenous regulators of cellular protein translation

34. Yes, because they have been conserved across species during evolution and study in pigs can provide information about microRNA in human IAV infection. But have to be interpreted cautiously because microRNA have a complex regulation

35. It refers to how animal model is a mirror of human clinical signs of disease

36. It refers to similarities for underlying pathogenic mechanisms of the disease

37. It refers to how accurately an animal model reflects the pharmacological effects of treatments related to humans

38. Those with high face and /or high predictive validity

39. F

40. T

41. b

42. T

43. T

44. Because in pigs are well described states that involves innate immune system, as low grade inflammation associated with obesity and aging, as can happen in humans.

**Liu and Ji.** [**Dietary Factors in Prevention of Pediatric *Escherichia coli* Infection: A Model Using Domestic Piglets**](https://academic.oup.com/ilarjournal/article/59/3/338/5490289)**, pp. 338-351**

Domain 3: Research; K3. animal models (spontaneous and induced) including normative biology relevant to the research (e.g., background lesions of common strains)

Primary Species: Pig (Sus scrofa)

SUMMARY: Pediatric diarrhea is a leading cause of infant mortality and morbidity, especially in developing countries (~ half million deaths worldwide, yearly). Of these, E. coli accounts for <40% of infections.

While many factors influence the epidemiology of these infections, dietary influences are significant. Exclusive breastfeeding is the single highest protective factor in infant diarrhea. However, which bioactive compounds within the milk provide the benefit are unknown, and other compounds may provide similar or superior benefit e.g. secretory antibodies, bactericidal enzymes, oligosaccharides, lactoferrin, phytochemicals.

A reliable animal model is required to mimic the conditions of administration. While rodents are the most widely used biological model, their anatomical and immune responses are very different. Rodents are not suspectable to endotoxemic E. coli infection, whereas pigs are. The non-human primate infant may be used as a model, but these animals present ethical and practical challenges.  Pigs are readily available and have similar immune systems and GI tracts compared to humans. The physical anatomy of the GI tract is similar (glandular stomach, sacculated colon) and the GI flora are broadly similar. Neonatal swine are precious and readily adapt to artificial feeding systems which allows easy manipulation of the diet.

However, the piglet is not the perfect model as while there is broad similarities between humans and pigs, there are also significant differences including maternal antibody transfer, GI length and the presences of a spiral colon. Although the microbiome contains very similar species of bacteria, the ratios in which these are present in humans and pigs is very different.

Neonatal pigs were fed a variety of bioactive compounds with possible protective effects, and the challenged with ETEC.

QUESTIONS

1. How many layers comprise the porcine placenta?
2. What is the difference between prebiotics and probiotics?
3. What is lysozyme?

ANSWERS

1. 6
2. Prebiotics – compounds ingested to improve the health/ composition of the GI microbiome. Probiotics – bacteria ingested to change the health/ composition of the GI microbiome
3. Antimicrobial enzyme produced by animals that forms part of the innate immune system. Found in many bodily fluids e.g. milk and saliva, variation in this may partially account for susceptibility changes between formal and breast fed infants. (Human breast milk lysozyme = 390mg/l. Cow’s milk = <0.45mg/l)

**Gilger.** [**Immune Relevant Models for Ocular Inflammatory Diseases**](https://academic.oup.com/ilarjournal/article/59/3/352/4885866)**, pp. 352-362**

Domain 3: Research; K3. Animal models

SUMMARY: Dry eye and uveitis are common causes of blindness in both humans and domestic animals.  The eye, like the brain and the uterus in pregnancy, is considered an immune privileged site due to the blood-ocular barrier, lack of lymphatics, and the presence of limited numbers of resident leukocytes.  With dry eye and uveitis, physical barriers become disrupted, allowing an influx of inflammatory cells.  Proinflammatory mediators induce T-helper cells to proliferate and ultimately release proinflammatory and proapoptotic peptides.  Rodents are typically used to determine an appropriate dose for therapeutics.  Then rabbits, dogs, pigs, or primates can be used to determine if a drug is clinically feasible and to determine the pharmacokinetics and pharmacodynamics of a specific route of administration of a drug.

Dry eye is a disease of the tear film and ocular surface that develops from a deficiency of the aqueous portion of the tear fluid as a result of reduced lacrimal aqueous tear secretion or increased evaporation of tears (Meibomian gland deficiencies).  Decreased tear production results in an increase of tear electrolytes, proteins, and inflammatory mediators, resulting in damage to the surface ocular tissues, decreased visual acuity, and ocular discomfort.  Immunosuppressant treatments like topical cyclosporine inhibit T-cell activation and reduce proinflammatory cytokines.  Lifitegrast, a recently approved topical treatment for dry eye, is an integrin inhibitor that prevents binding of LFA-1 to ICAM-1, which is upregulated in dry eye disease.  Mouse models have been used to study the immunopathogenesis of dry eye and the initial evaluation of therapeutics.  Described models of dry eye include low humidity and high air flow environments +/- scopolamine, repeated application of topical benzalkonium chloride, lacrimal gland excision, or injections of toxins or antigens such as botulinum toxin or concanavalin A.  Genetic mouse models include the MRL/lpr or neurturin-deficient mice.  The most commonly described model in rats is the extraorbital lacrimal gland excision model, but this may not be as effective as naturally occurring models.  Rabbit or dog models are more commonly used to evaluate dry eye signs and response to therapy because they have easily measured decreased tear production and develop ocular surface changes.  Rabbit models include topical benzalkonium chloride, a short term reduction in lacrimal secretion using parasympathomimetic drugs like atropine, or intra-lacrimal or SC injection of autologous peripheral blood lymphocytes activated by purified rabbit lacrimal epithelial cells (produces a Sjogren’s-like keratoconjunctivitis).  Dogs develop spontaneous dry eye that clinically and immunopathologically is similar to dry eye in humans.  Canine dry eye is typically bilateral, develops in middle age, and is more common in female dogs.  Pathogenesis in dogs is similar to humans, where an inapparent immunologic inflammation occurs with progressive lymphocytic infiltration and damage to the lacrimal gland with subsequent decreased production of the aqueous tear film.

Uveitis is inflammation of the iris, ciliary body, and choroid and is associated with both infectious and noninfectious causes.  The uveal tract supplies blood to the eye and is in direct contact with peripheral vasculature, so diseases of the systemic circulation (septicemia, bacteremia, infection, activated lymphocytes, immune diseases, etc.) will disrupt the blood-ocular barrier.  Following disruption of the blood-ocular barrier, large amounts of predominantly CD4+ T cells enter the eye and secrete proinflammatory cytokines such as IL-2 and interferon γ.  Resolution of uveitis is dependent on the presence of T regulatory cells.  Rodents models of uveitis include endotoxin-induced uveitis with a IP, SC or hind footpad injection of endotoxin in Lewis or Sprague-Dawley rats or C3H mice (acute anterior uveitis).  Experimental autoimmune uveitis can be induced by immunizing susceptible rodents with retinal antigens (posterior uveitis) or by immunization with melanin (anterior uveitis).  Most of the rodent experimental models of uveitis are not recurrent.  Spontaneous uveitis has been observed in various mouse models, including IRBP T cell receptor transgenic mice (R161H) and autoimmune regulator (AIRE)(-/-) mice.  These mouse models have a gradual onset of chronic ocular inflammation that ultimately leads to retinal degeneration.  One advantage of rabbits models of uveitis over rodent models is that the rabbit eye is more similar in size to the humans eye.  The two most commonly evaluated rabbit models of uveitis include acute uveitis induced by injection of endotoxin and recurrent uveitis induced by tuberculosis antigen.  To stimulate chronic recurrent inflammation, eyes are re-challenged with intravitreal antigen every 14-21 days.  Unlike the rabbit, pigs have a retinal vascular anatomy similar to humans.  Endotoxin can be injected intravitreally and the eye monitored for up to 72 hours following injection.  Equine recurrent uveitis (ERU) is the most common cause of blindness in horses.  T cells are the predominant mononuclear inflammatory cells infiltrating ocular tissues in horses with naturally occurring chronic uveitis, with a significant number of CD4+ cells.  Studies of spontaneous ERU have helped elucidate the immunopathogenesis of recurrent uveitis.

Developing, targeted therapies for ocular disease include gene therapy and stem cell therapy.

QUESTIONS

1.   Which of the following is the most common mouse model of dry eye disease?

a.   Environmental chambers +/- scopolamine patch

b.   Botulinum toxin injection into lacrimal gland

c.  Intraorbital injection of concanavalin A

d.   Topical administration of benzalkonium chloride

e.   Extraorbital lacrimal gland excisions +/- scopolamine

2.  Which mouse model lacks lacrimal innervation and may serve as a model for neurotrophic keratoconjunctivitis sicca?

a.   MRL/lpr

b.   Neurturin-deficient

c. XID

d.  BALB/cByJ

e.   C3H/He

3.  Which of the following breeds does dry eye NOT commonly occur?

a.  American Cocker spaniel

b.   Bulldog

c.  West Highland white terrier

d.  Collie

4.   Which of the following is NOT a clinical sign of uveitis?

a. Corneal vascularization

b.  Flare

c.   Cell accumulation

d.   Vitreous haze

5.   Which cells are important to induce spontaneous resolution and in maintaining remission of uveitis?

a.  TH17 cells

b.  Interferon γ

c.   CD25+

d.  Foxp3+ Tregs

ANSWERS

1.  a – low humidity and high air flow environments

2.  b - MRL/lpr mice manifest multiple autoimmune disorders and can be helpful to study diseases such as SLE and Sjorgren’s syndrome

3.   d

4.  a – clinical sign of KCS

5. d