**ILAR J**

Volume 57, Number 1, 2016

***Models of Viral-Induced Carcinogenesis and Oncolytic Viruses***

**Lairmore and Niewiesk. Models of Virus-Induced Carcinogenesis and Oncolytic Viruses, pp. 1-2**

Primary Species: Mouse (Mus musculus)

Secondary Species: Cat (Felis catus domesticus)

Domain 3; K3

SUMMARY: A variety of infectious agents have been linked to cancer. Viruses, bacteria, and parasites have been linked to tumors in animals and humans. Viruses associated with cancer formation cause alteration of physiologic control of cell growth and proliferation or may increase the susceptibility of the host to other cancer risk factors such as environmental toxins. The study of virus–cell interactions has provided fundamental knowledge of cell biology and cell transformation. Viruses associated with cancer and model systems to study these mechanisms have established basic paradigms for virus–cell interactions and contributed greatly to the understanding of diseases associated with biomedically important viruses. More recently, some viruses, the so-called oncolytic viruses, have been used as agents against cancer. This issue of the ILAR Journal is dedicated to models of virus-induced carcinogenesis and oncolytic viruses.

QUESTIONS

1.  Which are true statement about a model for HIV-1 infection and disease that allowed the comparative study of lentivirus as a model of HIV-1 infection and disease?

a.  Domestic cats

b. Feline immunodeficiency virus (fiv) as a naturally occurring retrovirus

c. Nondomestic feline species

d.  All the above

2. One of the earliest viruses associated with cancer in the mouse?

3.  To understand the infection and pathogenesis of Human T-cell Leukemia Virus Type 1 (HTLV-1), what series of animal models have been used?

a.  The rabbit model of infection has been consistently used to study htlv-1 infection determinants and to test antiviral approaches such as vaccines.

b.  Transgenic mouse models to understand the pathogenesis of htlv-1–associated lymphomas

c.   New Zealand white rabbit model to study gut-associated diseases of humans.

d.  All the above

ANSWERS

1.  d. All the above

2.  Mouse mammary tumor virus (MMTV)

3.  d.  All the above

**Niewiesk. Animal Models of Human T Cell Leukemia Virus Type I Leukemogenesis, pp. 3-11**

Domain 3

SUMMARY:  This article summarized multiple animal models that have been developed in order to understand the development of leukemia and lymphoma induced by infection with human T cell leukemia virus type 1 (HTLV-1), a delta retrovirus.  Infection with HTLV-1 in humans leads to lifelong persistence, with a small percentage developing HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) or adult T cell leukemia (ATL).  Several animal models have been used to study the various stages of HTLV-1 infection, including the rabbit and mouse model of persistent HTLV-1 infection, transgenic mice to model tumorigenesis by HTLV-1-specific protein expression, ATL cell transfers into immunodeficient mice, and infection of humanized mice with HTLV-1.  The rabbit seems the most useful model to study chronic asymptomatic HTLV-1 infection due to the ease and consistency of viral transmission and infection.  Tax-and HBZ-transgenic mice are useful tools to study the contribution of those specific proteins, but one drawback is that the most effective promoter has to be used to express the proteins in CD4T lymphocytes.  The SCID mouse has been a successful model to investigate the proliferative and tumorigenic potential of ATL cell lines, and has also shown to develop humoral hypercalcemia of malignancy.  The Met-1 NOD/SCID-based mouse strain produces a cell line that metastasizes systemically and has been used to test measles virus virotherapy.  NOD/SCID mice have also been used to test drugs against HTLV-1 infection and tumor formation.  The use of humanized mice is still new, but they have been used to study the development of adult T cell leukemia after infection with HTLV-1.  It was proposed that humanized mice could enable investigation of the adaptive immune response and aid in developing vaccines against an infectious cancer in that model.

QUESTIONS

1.  Which of the following viruses is a member of the delta retrovirus family?

a.  Mouse mammary tumor virus

b. Human T cell leukemia virus

c. Murine Leukemia virus

d. Bovine leukemia virus

e. b and d

2. A model of HTLV-1-induced paraparesis has been established in which rat?

a. WKA

b. Athymic F344/N

c. LEW

d.  Wistar

3.  Which of the following mice do not have a functional B cell or T cell response, and reduced NK cell activity?

a.  SCID

b. Nude

c.  NOD/SCID

d. Rag-deficient

ANSWERS

1. e

2.  a

3.  c

**Dudley et al. Lessons Learned from Mouse Mammary Tumor Virus in Animal Models, pp. 12-23**

Domains 1 and 3

Primary Species: Mouse (Mus musculus)

SUMMARY:  Mouse Mammary Tumor Virus (MMTV) was first described in 1936 as a milk-borne agent that could cause cancer. One of the first instances of an oncogenic virus.  MMTV is a retrovirus.  It was later discovered that the MMTV provirus was incorporated into mouse genome (anywhere from 1-6 copies depending on the mouse strain).  Some mouse strains have mutations in the *mtv loci* and therefore a nonfunctional provirus is expressed, other strains express fully functional provirus (these strains have high rates of mammary tumors).  Milk borne infection is still possible.  It is not known to be able to infect human cells.

The primary target for the virus are dendritic cells and mammary epithelial cells.  Mice that lack Sag-cognate T cells are immune to infection of MMTV.  Typically 6 months to a year after infection a female mouse will be found with mammary tumors.  This is caused by insertional mutagenesis, rather than the virus itself carrying an oncogene.  Transgenic mice expressing either WNT1 or FGF3 under the control of the MMTV LTR showed multiple mammary hyperplasias.  Metastasis is possible with MMTV induced mammary carcinoma.

MMTV has historically been a helpful model in understanding some aspects of human breast cancer.

QUESTIONS

1.  Mouse Mammary Tumor Virus is transmitted to offspring in what way(s)?

a. Milk-borne transmission of virus

b.  Oral transmission from mother while cleaning after labor

c.  Fecal oral transmission

d.  Germline transmission

e.  a and d

2. MMTV enters the gut epithelium via what type of cell?

a. M cell

b.   Goblet cell

c. Paneth cell

d.  Enterocytes

3. MMTV causes mammary carcinoma in mice via what process?

a.  Delivery of the *myc* oncogene

b.  Activation of murine endogenous oncogenes

c.  Insertional mutagenesis

d.  Viral inactivation of tumor suppressor genes.

ANSWERS

1. e

2. a

3.  c

**Kaye et al. Role of Feline Immunodeficiency Virus in Lymphomagenesis – Going Alone or Colluding?, pp. 24-33**

Domain 1: Management of Spontaneous & Experimentally-Induced Diseases & Conditions

Secondary Species: Cat (Felis catus domesticus)

SUMMARY: Feline immunodeficiency virus (FIV) is a species-specific lentivirus of domestic and nondomestic felines. It is the only T cell-tropic pathogenic lentivirus other than HIV, but both viruses have the ability to infect other leukocyte and nonleukocyte cells. Due to its abilities to cause AIDS-like disease, it has been used as a model for HIV infection in humans almost since its discovery in 1986. In addition to AIDS-like syndrome, FIV has also been associated with the development of malignant lymphoma in cats, similar to what has been described for HIV and SIV in their host species. Of the two feline retroviruses, FeLV is by far more virulent and is directly oncogenic, whereas the mechanisms of FIV-induced lymphomagenesis is more likely an indirect mechanism that involves chronic lymphocyte activation and/or defective cell-mediated immunosurveillance.

In both humans and macaques, gamma herpesviruses (GHVs) have been implicated in the development of lymphomas in retrovirus-infected individuals. Almost all species within the gammaherpesvirinae subfamily are lymphotropic, establishing latent infections in lymphocytes, and many appear to be involved in the development of lymphoproliferative disorders (LPDs). The molecular mechanisms of GHV-induced oncogenesis may be tightly intertwined with the mechanisms of viral latency and its regulation, which would fit with the observation that many lymphomas appear to arise in tissue with chronic immune stimulation such as gastrointestinal tract and gut-associated lymphoid tissues. Most GHVs appear to give rise to LPDs, including lymphoma, only in the context of coinfection with a retrovirus, infection of a heterologous host, or immunosuppression, suggesting that host factors play an important role in driving lymphomagenesis.

Recently, three novel and species-specific GHVs were discovered in cats, with FcaGHV1 being the GHV found in Felis catus. Subsequent studies showed a likely worldwide distribution of FcaGHV1, with prevalence of infection varying from 5% up to 25-30% of cats in different geographical areas. Risk factors for FcaGHV1 infection include age, being male, and coinfection with FIV or FeLV, which suggests a strong link between FcaGHV1 exposure and male cat behavior and horizontal transmission as the predominant mode of infection. Although the detection of coinfections of pathogenic retroviruses and FcaGHV1 in cats with lymphoma is reminiscent of findings in other species with lymphoma, a causal relation in lymphomagenesis remains to be demonstrated for FcaGHV1, and FcaGHV1’s role in other diseases and malignancies remains unknown.

Lymphomagenesis in felines requires further investigation of the mechanism of oncogenesis of FcaGHV1 in the face of an FIV infection. It will be essential to obtain isolates of FcaGHV1, establish in vitro culture systems for the virus, and to further characterize the complete genome and assess whether regional variants exist. Assessment of the role of heterologous immunity in the pathogenesis of these viruses and the interactive role they may play in lymphomagenesis will also be necessary.

QUESTIONS

1. Feline immunodeficiency virus is a member of what species of viruses

a. Flavivirus

b. Lentivirus

c. Picornavirus

d. Polyomavirus

e. Cytomegalovirus

2. True or False: Of the 2 feline retroviruses, FIV is the more virulent than FeLV.

3. In humans and macaques, which subfamily of Herpesviridae is associated with oncogenesis and implicated in the development of lymphoma in retrovirus-infected individuals?

a. Alphaherpesvirinae

b. Betaherpesvirinae

c. Gammaherpesvirinae

d. Deltaherpesvirinae

4. True or False: Gamma herpesviruses are commonly transmitted across species.

ANSWERS

1. b

2. False

3. c

4. False, usually species-specific

**Haines et al. Characterization of New Zealand White Rabbit Gut-Associated Lymphoid Tissues and Use as Viral Oncology Animal Model, pp. 34-43**

Primary Species: Rabbit (*Oryctolagus cuniculus*)

Domain 3

SUMMARY: The objectives of this article included immunophenotypic characterization of populations and distributions of lymphocytes in rabbit gut associated lymphoid tissue (GALT.) The authors examined inductive and effector sites and compared them to other secondary lymphoid tissues and to GALT in humans. 12 week old SPF HsdOkd: NZW rabbits were saline perfused under anesthesia followed by euthanasia and collection of tissue samples from the mediastinal lymph nodes, Peyer’s patches, cecal tonsil, appendix, ileocecal plaque, and spleen. Intraepithelial lymphocytes (IELs), lamina propria lymphocytes (LPLs), and lymphocytes from blood samples were isolated and characterized via flow cytometry. Histologic analysis was also performed on the palatine tonsil, stomach, duodenum, pancreas, jejunum, ileum, jejunal and ileal Peyer’s patches, ileocecal plaque, cecal tonsil, appendix, cecum, colon, mesenteric lymph nodes, spleen, and liver. Immunohistochemistry was performed on frozen sections of the same tissues. A summary of results follows:

Histology: The majority of GALT was in the Peyer’s patches, appendix, and cecal tonsil. Diffuse populations of IELs and LPLs were present within the small and large intestine. Some animals had lymphoid aggregates in the stomach body, pylorus, cecum, or colon.

Lymphocyte subset quantification: T cell percentages were higher than B cell percentages in the mesenteric lymph nodes and IELs/LPLs. In the spleen, the majority of cells were B cells. In inductive sites (Peyer’s patches, cecal tonsil, ileocecal plaque), B and T cells were balanced and in the appendix, B cells predominated. CD4/CD8 T cell ratios were balanced in LPLs and IELS, but high in GALT inductive sites, the ileocecal plaque, the cecal tonsil, and Peyer’s patches as compared to ratios in cats, rhesus macaques, and humans.

Immunohistochemistry: Peyer’s patches, appendix, cecal tonsil, and ileocecal plaques, all contained prominent subepithelial B cell domes and follicles with germinal center surrounded by T cell rich zones. B and T cell ratios were similar in Peyer’s patches and ileocecal plaques, increased in cecal tonsils, and highest in the appendix. The Peyer’s patches, cecal tonsil, and ileocecal plaque were T cell dominant and most similar to the mesenteric lymph nodes in T cell population. The appendix was B cell dominant and most similar to the spleen in T cell population.

Germinal Center Formation: All inductive sites had germinal center activity, but this was significantly smaller in the Peyer’s patches (2 – 4x smaller)

Rabbits share many gastrointestinal similarities to humans, including in B lymphocyte populations in the rabbit appendix and ultrastructural and M cell similarities in the palatine tonsils and Peyer’s patches. This study indicates that the majority of GALT in the rabbit is present in the inductive sites. IELs and LPLs were present throughout the gastrointestinal tract and CD4+ T cells predominate, as in other species including humans. The cecal tonsil, which has previously been compared to the human appendix based on morphologic analysis, does not appear to have a similar lymphocyte population to the human appendix. The ileocecal plaque is essentially a large Peyer’s patch in a specific location and is most similar to the Peyer’s patches found at the ileocecal orifice of many mammals, including humans. The cecal tonsil has a similar lymphocyte distribution to Peyer’s patches, but unique B and T cell ratios.

QUESTIONS

1.   What is another name for the cecal tonsil in the rabbit?

2.  Which portions of the gut act as inductive sites?

3.  Which portions of the gut act as effector sites?

ANSWERS

1.  The sacculus rotundus

2.  Peyer’s patches, isolated lymphoid follicles, and lymphoglandular complexes in the large intestine. These include the appendix, the cecal tonsil, and the ileocecal plaque and may include the palatine tonsil in the rabbit.

3.  Dispersed memory phenotype lymphocytes including intraepithelial lymphocytes and lamina propria lymphocytes

**Hudson and Colvin. Transgenic Mouse Models of SV40-Induced Cancer, pp. 44-54**

Primary Species: Mouse (*Mus musculus*)

Domain 3 TT3.3

SUMMARY: SV40 viral oncogene was first used in 1974 to generate transgenic mice.  It works to inactivate two tumor suppressors, p53 and retinoblastoma, and large T and small t antigens work with proteins which enhance the ability of SV40.  There are 2 SV40-induced bladder cancer models.  One works to express large T antigen to the basal layer of the urothelium using uroplakin II.  The other used cytokeratin 19 gene regulator element to express SV40 large T antigen to urothelial cells.  There are several liver models.  One of the first used major urinary protein promoter to express coding region for large T and small t antigen to hepatocytes.   Another used the 5’ flanking region of the alpha-antitrypsin gene, serum amyloid P component promoter, and albumin enhancer.  The most popular uses antithrombin III gene to express large T and small t antigen in hepatocytes.  Most recently, there is an inducible Tag model with tumorigenesis occurring due to adenovirus delivery of Cre recombinase or tetracycline regulatory system.  Multiple models have been generated for ocular tumors using SV40.  One model uses the large T and small t antigen expression to gonadotropes using hormone beta-subunit promoter.  Other ocular models used human interphotoreceptor retinoid binding promoter expressed in rods and cone photoreceptors.  Nonspecific tumors have been developed by targeting amacrine neurons of the retina using phenylethanolamine N-methyltransferase promoter.  Using murine interstitial retinol binding protein resulted in 100% penetration.  In an oncogene model, alpha-crystallin A promoter was used for large T antigen to the lens.  Melanoma SV40 models are limited due to the lower frequency of occurrence; there are only 3 major models.  One model uses tyrosinase promoter to express large T and small t to melanocytes to create ocular and cutaneous hypopigmented tumors.  Using the same promoter another model developed with no cutaneous tumors.  Intestinal models have been developed to study proliferation; the Fabpi/SV40Tag model causes crypt tissue proliferation without tumor formation.  Intestinal carcinomas have been generated using Villin promoter to express SV40Tag and Tag mutants in the crypts.  A small cell carcinoma of the colon model was creased using intestinal trefoil factor promoter.  Two stomach models have been developed.  On was generated to target SV40 expression to committed gastric epithelial precursors resulting in hyperplasia and thickening of the stomach wall.  Another used carcinoembryonic antigen causing dysplastic crypts.  Three SV40 breast cancer models are commonly used.  The C3(1)/Tag model is used to understand mammary tumorigenesis.  The WAP-mutp53 model exhibits higher tumor grade, enhanced vascularization and increased metastasis than WAP-T.  Over 30 years of research using SV40 viral oncogene models has contributed to genetic manipulation knowledge and has been reliable for generating different tumor types.

QUESTIONS

1. SV40 viral oncogene works to inactivate which two tumor suppressors and works with which antigens?
2. Name 3 different body systems affected by SV40 viral oncogene.
3. Why are there only 3 melanoma SV40 models?

ANSWERS

1. Tumor suppressors: p53 and retinoblastoma; antigens: large T and small t
2. Retinoblastoma, melanoma (skin/ocular), intestine, stomach, breast
3. Melanoma SV40 models are limited due to the lower frequency of occurrence

**Hassan Ahmed and Baiocchi. Murine Models of Epstein-Barr Virus-Associated Lymphomagenesis, pp. 55-62**

Domain 1; Task 3

SUMMARY: The Epstein-Barr virus (EBV) is a B-lymphotropic gamma herpes virus associated with a number of malignancies. Most EBV-related cancers present complex medical management challenges; thus it has been essential to develop preclinical in vivo models allowing for the study of pathogenesis, prevention, and treatment of these diseases. Early in vivo models used nonhuman primates; however, such models were limited by the inability of EBV to achieve viral latency, availability, and cost. Immunodeficient mouse strains emerged as efficient models that allow for engraftment of human mononuclear cells and controlled evaluation of EBV-driven lymphoproliferative disease (EBV-LPD). By using highly immunodeficient strains of mice such as severe combined immune deficiency (SCID) and NOD/LtSz-scid ILrg−/− (NOG) mice, investigators have developed efficient platforms for evaluating pathogenesis of benign (HLH) and malignant (EBV-LPD) diseases associated with EBV. Humanized murine chimeric models have been essential tools for evaluating preventive strategies with vaccine and
adoptive cellular approaches, as well as development of experimental therapeutic strategies. Manipulation of the human immune cells before engraftment or mutation of viral lytic and latent genes has enhanced our understanding of the oncogenic nature of EBV and the complexity of human immune responses to EBV. In this review, we discuss how the EBV murine models have evolved to become essential tools for studying the virology of EBV as it relates to human EBV-LPD pathogenesis, the immunobiology of innate and adaptive responses, and limitations of these models.

QUESTIONS

1. Epstein-Barr virus belongs to which viral group:

a. Gamma 1 (lymphocrytovirus) herpesvirus

b. Gamma 2 (Rhadinovirus) herpesvirus

c. Flavivirus

d. Paramyxovirus

2. Which nonhuman primates are susceptible to experimental infection with EBV?

a. Callithrix jacchus

b. Saguinus oedipus

c. Macaca fascicularis

3. Several mouse models have been very useful for the study of EBV, these include?

a. NSG

b. NOG

c. BRG

d. All of the above

ANSWERS

1. a

2. a and b

3. d

**Speranza et al. Preclinical Mouse Models for Analysis of the Therapeutic Potential of Engineered Oncolytic Herpes Virus, pp. 63-72**

Domain 3

Primary Species: Mouse (*Mus muscularis)*

SUMMARY: This article is a review of the oncolytic herpes viruses (oHSVs) function as a therapeutic agent through direct oncolytic cancer cell-killing mechanisms and by stimulating the antitumor immunity and potential as an in situ anti-tumor vaccine. Oncolytic agents (herpes simplex virus type 1, adenovirus, reovirus, measles virus, vaccinia virus, and retrovirus) are delivered directly by injection into the tumor or systemically. They can be engineered to improve their therapeutic efficacy. In 2015 the first successful large, randomized, phase II clinical trial was conducted on advanced melanoma using talimogene hlaherparepvec (T-VEC), an engineered immunostimulatory oHSV. It resulted in improved durable response rates and was shown to stimulate antitumor T cell responses. The rapid infection and lysis cycle of HSV promoted its development as an ocoytic agent. To minimize effects on normal cells many gene deletions have been made.

OV Mode of Anticancer: infection in tumor cells with rapid replication and lysis leading to necrosis stimulating the cytotoxic T cell response against remaining cells.

*Immunocompromised Models*

* + Most commonly used is tumor implanted (SQ in flank or orthotopically) nude mice
	+ Patient-derived xenograft (PDX) model – fresh or low-passage patient-derived samples implanted directly into the flank of animals; predictive model; not yet reported in literature w/ oHSV
	+ Used for study of innate immune system (the resistant mechanisms to oHSV), and therapeutic combinations w/ various small molecules, specifically irradiation.
	+ Limitation in understanding interaction of oHSV w/ host adaptive immune system

*Immunocompetent Models*

* + Cross species barrier include human HSV infection leading to necroptosis in mice models.
	+ Early syngeneic mouse models were used to show immune stimulation by oHSV.

o   Limitations: replication of HSV-1 limited, poorly characterized genetically, doesn’t allow for development of microenvironment, and important differences between immune system function than humans

* Genetically engineered mouse models of human cancer: not highly utilized
* There are not currently any immunocompetent rodent models that allow testing of oHSV-1 by supporting its oncolytic activity driven by rapid replication

QUESTIONS

1.  What is the mode of anticancer action of oncolytic agents?

2.  What are some oncolytic agents? Which was used in a Phase II clinical trial in 2015?

3.  What is the most commonly used mouse model for testing of oncolytic agents?

ANSWERS

1.  Infection in tumor cells with rapid replication and lysis leading to necrosis stimulating the cytotoxic T cell response against remaining cells

2. Herpes simplex virus type 1, adenovirus, reovirus, measles virus, vaccinia virus, and retrovirus: T-VEC (an oHSV)

3. Nude mice

**Falls et al. Murine Tumor Models for Oncolytic Rhabdo-Virotherapy, pp. 73-85**

Primary Species: Mouse (Mus musculus)

Domain 3 T3.3

SUMMARY: Oncolytic viruses can be selected to kill cancer cells.  Vesicular stomatitis viruses are rhabdoviruses that are highly sensitive to interferon.  Subcutaneous models are the most common, used for breast, colon, melanoma, and renal cancer cell lines.  Intravenous administration allows for colonization of lungs and hematologic models.  There are limitations with this route include volume that can be injected.  This can be overcome with various kinds of pumps through a catheter.  Intracardial tumor cell administration allows bypass of the lungs to infect other areas such as bones or brain.  Intranasal injections are easy to perform, but animals need to be anesthetized to decrease sneezing and thus loss of virus.  Direct organ injection is also a precise delivery system for specific organ infection.  The use of immunocompromised animals helps decrease rejection as well as lowers the lethal dose.  These strains come with their own challenges, such as increased surgery/anesthesia complications and infection.  Matrigel or cultrex can be coinjected to stimulate tumor growth.  There are various techniques to analyze tumors.  Titration uses plaque assay.  qPCR counts genome copies, not necessarily infection.  Bioluminescence and fluorescence imaging uses variants that express fluorescent proteins to measure expression of reporter transgenes.  A similar approach uses radioactive iodine with PET/CT scans.  IHC uses antibody against virus and takes longer to perform, but can localize the virus within the tumor or tissue.  Kupffer cells are specialized liver macrophages that work to deplete virus and in order to overcome this, clodronate liposomes can be used to deplete Kupffer cells.  One risk with using systemic infection is toxicity associated with infection of normal tissues.  Knockout mice can provide information on virus clearance.  PKR-/- mice lack IFN antiviral response and are hypersensitive to VSV infection.  IFN alpha and beta receptor null mice have also been used, along with the STAT1 knockout mice.  Certain combination strategies have been employed to improve the efficacy of OV therapy.  One strategy is to combine radiotherapy or chemotherapeutics with VSV.  Another strategy is to combine VSV with vaccination.   Irradiated virus infected cells have also been used, improving efficacy.

QUESTIONS

1. Vesicular stomatitis viruses are in what family of virus?
2. Intracardiac tumor cell administration allows bypassing of what organ?
3. What are Kupffer cells?

ANSWERS

1. Vesicular stomatitis viruses are rhabdoviruses that are highly sensitive to interferon
2. Intracardial tumor cell administration allows bypass of the lungs to infect other areas such as bones or brain
3. Kupffer cells are specialized liver macrophages that work to deplete virus and in order to overcome this, clodronate liposomes can be used to deplete Kupffer cells

**Lewis et al. Institutional Animal Care and Use Committee Considerations Regarding the Use of Virus-Induced Carcinogenesis and Oncolytic Viral Models, pp. 86-94**

Domain 5: Regulatory Responsibilities

SUMMARY: Viral vaccines and genetically engineered viruses have been proposed as targeted therapies to kill tumors.  The anticancer activity of oncolytic viruses stems from the ability of these viruses to infect and kill tumor cells directly and/or to target the tumor vasculature, which causes indirect death of tumor cells.  Research using oncoviruses in animal models has provided important information regarding basic cell biology and disease mechanisms, resulting in important interventions for the control of human cancers.  Although insights gained from in vitro models is invaluable, the use of an in vivo animal model is essential to the understanding of the complicated interaction between tumor and virus.  The dichotomy of animal welfare in cancer research is that animal models are developed and used to test the efficacy of potential life-saving agents against a fatal and painful disease.  The use of in vivo cancer models is a privilege that requires special considerations from the IACUC.

IACUCs may request pilot studies in some circumstances where knowledge regarding the proposed model, interventions, or procedures is sparse or unavailable.  When unique transgenic mouse strains are generated for cancer studies, such as humanized mice, careful observation for signs of abnormalities that impact animal health should be required.  Strain phenotype evaluations should be reviewed during post approval monitoring.  Collaboration between a veterinarian and the principal investigator will ensure that protocols reflect appropriate selection of anesthesia/analgesia to meet clinical and humane requirements as well as the needs of the research protocol.

Cancer models include either those using tumor cell transplantation or those in which tumors arise or are induced in the host.  Subcutaneous injection of tumor cells in the flank is one of the most common mouse tumor models used in cancer research.  Internally growing tumors, such as orthotopic tumors, or tumor-associated metastatic disease can be particularly challenging because there is not an obvious tumor to measure.  Monitoring the development of isolated, well-demarcated solid tumors is relatively straightforward.  Tumor burden should be limited to the minimum volume necessary to achieve scientific results.  Recommended mean size of single tumors should not exceed 1.2 cm in mice or 2.5 cm in rats.  Exceptions to these size limits require justification and should be part of the IACUC considerations during protocol review.

Animal management and personnel safety should be addressed carefully in the IACUC protocol.  Information concerning animal housing, handling, and humane euthanasia of irradiated animals, survival surgery, and survival studies should be addressed.  If individual housing is required, it should be scientifically justified and environmental enrichment should be provided whenever possible.  The animal model, type of virus, and how it is used determines what safety considerations are important.  Safety issues with oncolytic viruses are considered to be similar to those with live-attenuated viral vaccines. The most common level of biocontainment used to isolate potential hazards in cancer research is BSL-2.

QUESTIONS

1. Selection of the type of tumor model should be based on:

a. The molecular characteristics of cancer cells

b. Rate and reproducibility of tumor growth

c. Metastatic potential

d. Sensitivity to treatment

e. All of the above

2. True or False: The choice of site for tumor injection is a determining factor for how many cells can be injected (total volume) and how large the tumors can be allowed to grow.

3. The incidence, site of origin, growth rate of the experimental tumor and the onset and nature of any associated adverse effects will vary with all but which one of the following?

a. The virus used to induce the tumor

b. The biology of the tumor

c. The site of development of the tumor

d. The type of gloves worn in the facility

e. The host response to the tumor

4. Common clinical assessments of internally growing tumors include all but which one of the following?

a. Palpation to determine tumor growth

b. Use of calipers measuring two dimensions at right angles

 c. Palpation to determine organ enlargement

     d. Evaluation of respiratory patterns for metastatic lung disease

    e. Serial diagnostic imaging

5. True or False: Body condition scoring and/or weight loss measurement can be useful when evaluating the general health of the animal.

ANSWERS

1. e

2. True

3. d

4. b

5. True