Chapter X Section G

Primate Late Effects Methods

J Mark Cline, DVM, PhD, DACVP
Professor of Pathology/Comparative Medicine
Wake Forest School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157
Office 336 716 1564
jmcline@wakehealth.edu

Gregory O Dugan, DVM
Assistant Professor of Pathology/Comparative Medicine
Wake Forest School of Medicine

Background and Introduction

This review will focus primarily on late effects of total body irradiation (TBI) in nonhuman primates (NHP). To the degree that acute effects inform this topic, they well be discussed as well.

NHP Species Studied

In general the abbreviation “NHP” will refer to rhesus macaques (*Macaca mulatta*), however, data regarding other primate species have been included. Most work in the field has been done in Asian macaque species, primarily the rhesus macaque but less often the cynomolgus macaque (*Macaca fascicularis*), a closely-related coastal relative, and rarely the pigtailed macaque (*Macaca nemestrina*). All macaques are outbred and have high inter-individual genetic variability, and most share a high degree of genetic similarity with the human primate, i.e. 93.5% (Rhesus Macaque Genome Sequencing and Analysis Consortium, 2007) compared with 67% in the laboratory mouse (*Mus musculus*).

The Acute Radiation Syndrome in NHP

The TBI Acute Radiation Syndrome (ARS) in NHP has been thorougly described by several investigative groups, and dose-response relationships are well-known although variable by laboratory (Allen et al., 1960; Macvittie et al., 2012). In the TBI model in the short term (days to weeks) mortality results from hematopoietic injury through leukopenia or thrombocytopenia with an LD50/30 of approximately 7 Gy. Thus, in NHPs higher-dose studies require partial-body exposures. The next dose threshold is pneumonitis occurring roughly 100 days after exposure at whole-thorax doses over 10 Gy, and beyond that, the gastrointestinal (GI) syndrome which has a fulminant course including an LD50/15 of 11 Gy without bone marrow sparing or 12 Gy with %5 bone marrow sparing (Macvittie et al., 2012). Acute single-dose central nervous system mortality in NHP occurs with an LD50 of 20 Gy within a few hours (Allen et al., 1960).

Late Effects

The major public health burden of radiation-associated disease is due to late effects. Virtually every organ is affected to some degree by oxidative and pro-inflammatory systems activated throughout the body. Circulating inflammatory mediators (e.g. monocyte chemoattractant protein 1, MCP1 and lipopolysaccharide binding protein 1, LBP) are persistently elevated in NHP, for up

to 10 years following irradiation at 6.5-8.5 Gy (Debo et al., 2016). This type of system-wide change cannot be definitively attributed to a single organ system; although impaired GI barrier function is likely a major contributor (MacVittie et al., 2012), the relative roles of other factors such as persistent immune impairment and long-term vascular endothelial dysfunction remain to be determined. Both systemic and local tissue derangements are clearly evident in some organ systems. We have shown through long-term longitudinal assessments that patterns of co-morbidity over time follow a quadratic curve, with the likelihood of multiple organ impairment increasing exponentially with dose (Cline et al., 2016).

Sources of Background Variation in NHP Studies

Baseline Data Acquisition and Experimental Control

A key advantage of studying nonhuman rather than human primates is that their exposures are planned, and thus pre-existing variability can be controlled for. Animals entering acute or long term studies should be carefully selected, with consideration of pathogen status, age, and sex. Experimental variables such as exposure to dietary radioprotectors can be controlled. Screening tests can be used to exclude animals with pre-existing disease.

NHPs included in long-term radiation effect studies are unavoidably a selected population, and increasingly so as exposure doses increase. The LD50/60 for 6MV linear accelerator-derived photons at 80 cGy/min is approximately 7 Gy for total body exposure with supportive care (MacVittie et al., 2012), with the LD10/60 estimated at 6 Gy. Any study of these survivors must include an assessment of those factors that permitted their survival, whether those factors are intrinsic (e.g. genetic, epigenetic) or extrinsic (e.g. environmental, infectious).

Pathogen Status

In general, all NHPs are maintained free of tuberculosis (TB) and simian immunodeficiency viruses (SIV), unless intentionally infected in a controlled setting. Beyond that base level, 4 SPF levels have been defined by the Institute for Laboratory Animal Research (Morton et al., 2008) to exclude other viral pathogens such as simian retroviruses which are immunosuppressive and associated with mesenchymal neoplasms (SRV); simian T-lymphotrophic viruses which cause variations in lymphocyte numbers and are associated with lymphomas (STLV); and macacine herpesvirus 1 or “B virus”, an alphaherpesvirus which is of little consequence to macaques but is a devastating neurotrophic pathogen in exposed humans (MHV). These are summarized in Table 1. Additional viral pathogens may be controlled for in some populations designated “Level 4 SPF”, for example cytomegalovirus (RhCMV), or the Epstein-Barr-like rhesus lymphocryptovirus (RhLCV). However, in the absence of this designation it should be assumed that most macaques are infected with RhCMV and RhLCV.

Table 1. Specific Pathogen Free Categories for macaques. Modified from Morton et al., 2008.

SPF Level	TB	SIV	SRV	STLV	MHV	Other
1	X	X	X			
2	X	X	X	X		
3	X	X	X	X	X	
4	X	X	X	X	X	X

Common pathogens usually not controlled for and likely present in the “background” of irradiated NHP include:

- Simian Varicella Virus - This common alphaherpesvirus produces acute multisystemic infections in young animals, and recurrent cutaneous infections in aged animals, much like Varicella-Zoster in humans. Notably, Simian Varicella “breaks” have been reported in irradiated macaques (Kolappaswamy et al., 2007; Hukkanen et al., 2009; Gulani et al., 2016).
- Cytomegalovirus - this betaherpesvirus affects most macaques and can produce serious infections in immunosuppressed animals.
- Lymphocryptovirus - this gammaherpesvirus affects most macaques, and causes an acute “mononucleosis”-like infection (Moghaddam et al., 1997) with lymphocytosis and lymphadenopathy, and B-cell lymphomas (Marr-Belvin et al., 2008).
- Papillomaviruses - A variety of genital and cutaneous papillomaviruses are highly prevalent in macaques and are associated with both benign and malignant neoplasms, as in many species (Wood et al., 2004, 2007).
- Pathogenic intestinal bacteria - Campylobacter, Salmonella, Shigella and others. These common pathogens are difficult to completely exclude.
- Streptococcus pneumoniae - A non-commensal respiratory pathogen in macaques, resulting in sporadic pneumonia (Philipp et al., 2012)
- Staphylococcus aureus - A ubiquitous cutaneous bacterium, often responsible for wound infections. Methicillin-resistant Staph aureus (MRSA) infections have been reported in macaques (Kolappaswamy et al., 2008).

Age and Sex

Sensitivity to ionizing radiation is higher in young individuals (IARC, 2000), and the distribution and proportion of radiation-sensitive proliferating cells is different in young animals compared to adults. Some critical tissues such as the brain, thymus, long bone growth plates, and gonads are orders of magnitude more radiation-sensitive in juveniles, relative to adults. Thus it is imperative to precisely define the age of NHPs used in radiation studies. Unfortunately this has historically not always been the case, with animals sometimes described only by body weight

Sexual maturity (reproductive competence) occurs at around 3.5 years of age in rhesus macaques, but mature body size is not reached until around 7 years of age (Cupp and Uemura, 1981). Age of animals purchased from commercial vendors may sometimes be misrepresented; therefore objective aging by dentition is necessary. Macaques have the same dental formula as humans (deciduous, 2 1 0 2; permanent, 2 1 2 3). An easily-assessed benchmark is the eruption of adult canine teeth, which in general corresponds to early adulthood at 3-5 years of age (Wang et al., 2016). Earlier ages can be estimated by the pattern of incisor eruption. Another “dental benchmark” is the eruption of the 3rd molars, which occurs at 8-9 years of age.

Study of both male and female animals is important to understand the impact of radiation injury on a population basis. Weight and stature of male and female macaques differs substantially, with males having a larger and more variable adult stature (Cupp and Uemura, 1981), as is the case with humans. Also important is the reproductive similarity between macaques and human beings. Only human females and old-world primates menstruate, and there is a corresponding increased erythropoietic requirement in females relative to males in both women and female macaques. Stress, whether physical or psychosocial, has profound effects on the hypothalamo-

pituitary-gonadal axis in both macaques and humans. Rhesus macaques are seasonal breeders in the wild, and this seasonality is maintained in captivity, with suppression of both male and female reproductive function in the summer (Bansode et al., 2003; Du et al, 2010).

Diet

The potential cofounding effect of dietary radioprotectants such as the phytoestrogen and tyrosine kinase inhibitor genistein has been known for many years (Landauer et al., 2003; Thigpen et al., 2004). For this reason studies of radiation typically use an open formula defined diet such as AIN93 (TestDiet, St. Louis, MO) (Reeves et al., 1993). Diets routinely fed to nonhuman primates have the same of high and highly variable genistein content; therefore the same degree of caution in dietary selection is indicated. Studies at Wake Forest make use of a “Typical American Diet (TAD)” developed in-house and now available commercially (Diet 5LOP, TestDiet, St. Louis, MO). This approach has the advantage of avoiding certain known radioprotectants, and providing an exposure to dietary macro and micronutrients that is similar to that of the human population.

Clinical Assessments of Late Effects

For the acute hematopoietic syndrome, clinical and pathologic outcomes are well-defined. For late effects, the spectrum of disorders is multisystemic, and continues to expand. Furthermore, as with human long-term cohorts, it is likely that a time by dose interaction is present, leading to organ system pathology at doses lower than expected. Therefore a thorough, unbiased, surveillance-based, multisystems approach is necessary, rather than an approach focused on expections. The following outcomes and observation frequencies are recommended (Table 2).

Table 2: Recommended circumannual frequency of clinical assessments for long-term effects in NHP.

Frequency	Daily	Monthly	Quarterly to Semiannually	Annually	As needed
Observation	X				
Physical Examination		X			
Blood Pressure					
Blood collection (archive)		X			
Urine collection (archive)		X			
Body weight		X			
Complete Blood Count			X		
Serum Chemistry Panel			X		
Hemoglobing A1c			X		
Echocardiography				X	
Flow Cytometry (PBMC phenotype)				X	
Bone marrow aspirate/viable freeze				X	
Computed tomography scan (whole body)				X	
Magnetic Resonance Imaging (head)				X	
Abdominal ultrasound				X	
Endoscopy/biopsy				X	
Bronchoalveolar lavage				X	
Ocular examination				X	
Biopsy (e.g. mass, skin lesion)					X
Glucose Tolerance Test					X

We have developed threshhold criteria for the diagnosis of common patterns of chronic morbidity, as shown below (Table 3).

Table 3. Criteria for threshhold-based diagnoses of morbidity during long-term observation of rhesus macaques.

Organ/Disease	**Criteria for Diagnosis**
Diabetes	Hemoglobin A1c (HbA1c) >6.5% Three fasting blood glucose measurements > 100 mg/dL Any non fasted blood glucose > 200 mg/dL
Urinary Tract	BUN > 30 mg/dl or Cr > 1.1 mg/dl Loss of renal volume >50% Urolithiasis
Lung	Pulmonary consolidation on CT scan Bullous emphysema >25% of lung volume Hypoxia under sedation (SPO2 < 80% or requiring oxygenation) Respiratory rate >80 breaths per minute
Gastrointestinal Tract	Any lesion on endoscopy Chronic diarrhea (severity code >2 for >5 days)
Skin	Persistent dermatitis, alopecia or loss of pigmentation
Cardiovascular System	Murmur detected on ausculation Valvular insufficiency on echocardiograph Mean arterial pressure >120 Stroke volume <5 mls/stroke & Cardiac output <0.5 L/min at >7 y
Brain	Y/N MRI lesions visible on SWI Neurologic abnormality on clinical exam
Cataracts	Any lens opacity on annual slit-lamp exam
Behavior	Any behavioral abnormality requiring management (customized environmental enrichment plan or medication)
Neoplasia	Any neoplastic disease (imaging or biopsy)
Testicular atrophy	Testis volume <10 ml each after 7 years of age; Vol = (p/6)(LxW^2)
Ovarian dysfunction	Absence of menstruation during winter LH > 30 ng/ml Or FSH > 3 ng/ml
Bone	Bone mineral content (BMC) < 274g Bone mineral density (BMD) < 0.373 g/cm^3 (- 2 SDs) (Excluding animals <7 yrs old). Fracture or other gross abnormality on clinical exam or CT scan
Abnormal Body Composition	Obesity: Waist Circumference > 45 cm or Dexa Body Fat > 30% Underweight: Waist Circumference < 25 cm or Dexa Body Fat < 12.3% (Excluding animals under 7 yrs old)

Post-mortem Assessments

All NHPs used in research should undergo a full gross and histologic post-mortem examination at the time of death, whether they are euthanized for experimental or humane reasons, or die unexpectedly. It is experimentally and ethically necessary, in order to maximize the experimental value of the animals, to understand the underlying causes of morbidity and mortality, and to reduce unnecessary suffering in future studies by identifying mistakes and opportunities for improvement. The causes of death in both acute and long-term studies are not always predictable, and may be multifactorial. Infectious disease must be identified when present. Stochastic events leading to death must be identified.

Standards for necropsy examination of NHPs are necessarily high, and should include the following elements:

- Review of the clinical history and experimental procedures
- Use of appropriate personal protective equipment and biohazard containment procedures
- Hematology prior to euthanasia, including a complete blood counts, blood smear, and if possible flow cytometric assessment of lymphocyte subsets
- A complete serum chemistry panel
- Blood cultures as indicated
- Gross examination and documentation of lesions
- Measurement of organ weights
- Systematic, documented collection of fixed and frozen tissues from all major organ systems
- Special tissue collections as resources permit (e.g. viably-frozen bone marrow, or special fixation for preservation of nucleic acids)
- Histologic examination of all major organ systems and any gross abnormalities
- Generation of a gross and histologic necropsy report
- Archiving of frozen and paraffin-embedded tissues

Published guidelines for necropsy examination of nonhuman primates may be useful (Wilson, 1985), but are generally forensically oriented and invariably require customization for each experimental setting. Consultation with a veterinary pathologist experienced in primate work is essential. The necropsy protocol used for the CMCR Radiation Survivor Core is available on request (jmcline@wakehealth.edu).

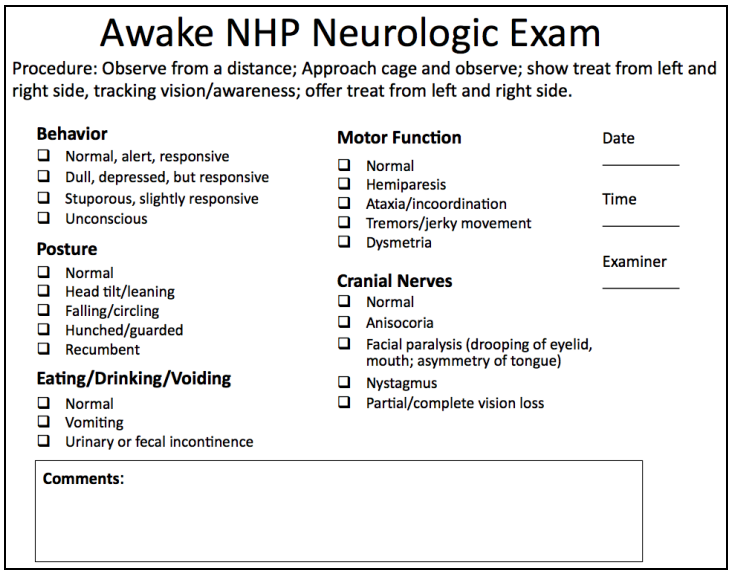
Observed Late Effects in Nonhuman Primates

Central Nervous System

Pathologic late effects of single-dose whole-brain irradiation of NHP include characteristic patterns of white matter degeneration and irregular vascular and perivascular structural changes leading to multifocal cerebral infarction (Caveness, 1977), as well as perivascular and diffuse cellular inflammatory changes and microglial activation (Price et al., 2001). Brain infarcts were seen within 6 months at single brain-only exposures above 15 Gy (orthovoltage, 250 kvp - Caveness, 1977). Similar structural changes are seen in fractionated exposures as well in NHP (Hanbury et al., 2015). Our laboratory has used the fractionated brain tumor therapy model for mechanistic studies of gene expression patterns (Andrews et al., 2017), and we have evidence

of incident brain lesions years after exposure detected by MRI in animals exposed to as low as 6.5 Gy (Cline et al., 2016), well below the previously reported threshhold for brain injury.

At high doses, injury to the CNS is usually but not always accompanied by clear neurologic signs. Simple cognitive testing strategies can be used to assess more subtle or higher-order degrees of neurologic impairment (Hanbury et al., 2015). The necessity for sedation to safely work with NHPs makes cooperative neurologic examinations in awake animals impractical. However, NHPs can be trained through positive reinforcement to perform some motor tasks, and a simple “cageside” neurologic exam is possible in awake animals. A typical worksheet for this type of examination is shown in Figure 1.



Awake NHP Neurologic Exam

Procedure: Observe from a distance; Approach cage and observe; show treat from left and right side, tracking vision/awareness; offer treat from left and right side.

Behavior
- ❑ Normal, alert, responsive
- ❑ Dull, depressed, but responsive
- ❑ Stuporous, slightly responsive
- ❑ Unconscious

Posture
- ❑ Normal
- ❑ Head tilt/leaning
- ❑ Falling/circling
- ❑ Hunched/guarded
- ❑ Recumbent

Eating/Drinking/Voiding
- ❑ Normal
- ❑ Vomiting
- ❑ Urinary or fecal incontinence

Motor Function
- ❑ Normal
- ❑ Hemiparesis
- ❑ Ataxia/incoordination
- ❑ Tremors/jerky movement
- ❑ Dysmetria

Cranial Nerves
- ❑ Normal
- ❑ Anisocoria
- ❑ Facial paralysis (drooping of eyelid, mouth; asymmetry of tongue)
- ❑ Nystagmus
- ❑ Partial/complete vision loss

Date _____

Time _____

Examiner _____

Comments:

Figure 1. Worksheet for neurologic examination of nonhuman primates in their home environment.

Cardiovascular System

Relatively low numbers of NHPs are under observation for cardiac “events” in the experimental setting. Such events may be fatal and thus result in loss of experimental data. Therefore, assessment of cardiac injury requires longitudinal measurement for detection of subclinical disease, and comparison to a control group. Diastolic dysfunction due to progressive myocardial fibrosis appears to be the typical pattern of long term injury (Debo et al., 2016). The post-mortem prevalence of myocardial fibrosis is nearly 50% in irradiated animals, in contrast to no fibrosis in control animals (Debo et al., 2016).

Measurement of cardiac dysfunction in NHP should include use of a clinical-grade ultrasound unit, with (at a minimum) measurement of systolic and diastolic left ventricular diameter, fractional shortening, ejection fraction, aortic diameter and LV outflow velocity. Blood pressure and body weight should be obtained at the time of echocardiography.

Diabetes

Type 2 diabetes mellitus (T2DM) is a late effect of TBI in human beings and rhesus macaques. In addition to routine body weight, anthropometrics, serum glucose, and whole blood hemoglobin A1c measurements, more detailed assessments can be used to evaluate peripheral

insulin resistance, including intravenous glucose tolerance tests and homeostatic modeling (Kavanagh et al., 2015). Central adiposity and lean body mass can be measured from CT scans (Kavanagh et al., 2015) or by dual energy x-ray absorptiometry (DEXA) (Summers et al., 2012)

Respiratory System

Pulmonary injury in rhesus macaques occurs at doses of 10-11 Gy and must be delivered as a partial-body exposure to avoid fatal ARS. Radiation pneumonitis occurs in most exposed animals at 2-3 months after irradiation, with a peak incidence at around 90-100 days (Garofalo et al., 2014). As the model is currently used, the effect of cardiac and thymic irradiation cannot be dissociated from the pulmonary exposure, and right ventricular enlargement secondary to pulmonary inflammation and fibrosis occurs in some animals (Cline et al., 2016). Study designs may or may not include treatment with antibiotics, corticosteroids, or novel mitigators. The most reliable clinical indicator of pulmonary injury is respiratory rate. Whole-chest, high resolution CT imaging may be used to assess the severity of acute or chronic disease. Pathologically, we have observed three main cellular processes contributing to pulmonary density: inflammation, which is primarily histiocytic; fibrosis, consisting of progressive patchy collagenous thickening of alveolar walls; and epithelial hyperplasia with varying degrees of metaplasia and dysplasia. Individual variation in response is substantial, which provides an opportunity to explore underlying mechanisms.

Gastrointestinal System

The gastrointestinal syndrome has both an acute aspect which cannot be dissociated from the hematopoietic component of the ARS, and a chronic aspect which is also difficult to define and liikely contributes to the persistent pro-inflammatory state in radiation survivors.

More to follow

Plasma citrulline concentrations can be used as a biomarker of acute gastrointestinal injury in rhesus macaques. Jones et al demonstrated that normal plasma citrulline in this species is ~40 uM, and drops within one day, reaching to a 50% nadir one week after 5% bone marrow sparing single-dose irradiation at 7.5-13 Gy. Loss of plasma citrulline in this study correlated with histologic injury, and although some animals in this study returned to normal or supranormal concentrations of citrulline, the longer-term biomarker use and predictive value of this marker are not strong (Jones et al., 2015).

Neoplasia

Studies of cancers in NHP have high value for late-stage development of molecularly-targeted therapies and imaging modalities, because NHP are genetically and physiologically similar to human beings. A few spontaneous and induced NHP neoplasms have been developed as translational models, with a well-characterized pathogenesis matching the human disease in a relevant way. For example breast cancers occur later in life and are usually hormone dependent as in women (Wood et al., 2006); cervical cancers are papillomavirus-induced (Wood et al., 2004, 2007; Chen et al., 2009); and B-cell lymphomas are associated with lymphocryptovirus infection (Rivallier et al., 2004; Marr-Belvin et al., 2008).

Attribution of carcinogenic risk requires either large numbers of individuals under observation, or unequivocally strong effects. Given that only tens of thousands of NHP are under medical observation worldwide, caution is indicated. However, the background prevalence of neoplastic

disease in NHP has been the subject of several recent reviews (Simmons and Mattison, 2011; Miller 2012), providing a baseline spectrum of disease against which signature neoplasms may be evident. Generally speaking, the most commonly diagnosed malignant neoplasms are ileocecal/colonic adenocarcinomas, and lymphomas; and some uncommon neoplasms have only rarely been reported outside the context of prior irradiation.

Broerse et al reported the most comprehensive long-term observational study of cancer incidence in irradiated rhesus macaques to date, including both orthovoltage and mixed neutron/gamma exposures and following the animals for 30 years, nearly their entire lifetime, in comparison to age matched controls (Broerse et al., 2000). Irradiated animals were likely prepubertal at the time of exposures (age estimated at 3 years and body weight 2.5-3 kg). Orthovoltage exposures (n=20) ranged from 2.8 to 8.4 Gy (mean 6.8 Gy), given at 300 kvp and 0.3 Gy/min. Neutron/gamma exposures (n=9) ranged from 2.3-4.1 Gy (mean 3.4 Gy), given at 0.08 Gy/min, consisting of 24% gamma irradiation. Twenty-one age-matched controls were also evaluated. In this long-term study, the relative risk (RR) attributed to x-ray exposure was 8-fold (95% CI 5-15) with an estimated excess RR/Gy of 1.1 (95% CI 0.5-2.1); for neutron exposures, the corresponding RR was 14 (95% CI 7-28), and the ERR/Gy was 3.8 (95% CI 1.8-8.0). Notably, the spectrum of tumor types differed in irradiated animals. Among the 21 controls 7 neoplasms were seen, of types commonly seen in rhesus monkeys (gastrointestinal and reproductive system carcinomas) with the most common being colonic carcinoma. In contrast, the irradiated animals developed renal cortical carcinomas, osteosarcomas, malignant vascular tumors, gliomas, schwannomas, and other mesenchymal tumors. Mean age of tumor onset was younger (15 years, compared to 29 years for controls). Average tumor number/animal was 1 for controls, 1.6 for x-irradiation, and 1.9 for neutron exposure.

Other investigators have reported potential radiation-associated neoplasms including sarcomas and glioblastoma multiforme (Kent and Pickering, 1958; Lonser et al., 2002). A particularly remarkable finding is the induction of glioblastoma multiforme in 9/12 juvenile rhesus monkeys given fractionated whole brain irradiation (3.5 Gy/fraction x 10, Co60, 70 cGy/min), arising 2-8 years after exposure (Lonser et al., 2002).

The data summarized here show clearly that the lifetime risk of neoplasia is substantially increased in irradiated NHP; that the risk greater for neutrons than x-rays; and that there is a “radiation signature” in the type of malignancies found, with renal and mesenchymal neoplasms being characteristic. Similar patterns of tumor incidence and type are occurring the the CMCR Radiation Survivor Cohort (Cline et al., unpublished data, RRS 2016).

Reproductive System

Testis and ovarian effects

More to follow

Endometriosis (Fanton and Golden, 1991)

Immune System

More to follow

Lymphodepletion and acute immune impairment occur in macaques as in other species, including reactivation of the latent alphaherpesvirus infection Simian Varicella (Kolappaswamy et al., 2007; Hukkanen et al., 2009; Gulani et al., 2016).

Bacterial infections

Longer-term effects of immune injury

Conclusions

Assessment of late effects in NHP requires first a knowledge of the baseline characteristics of the animals under study (species, subpecies, sex, age); then confirmation of their specific pathogen status; then a thorough understanding of the experimental conditions to which they have been exposed. Given the high value of the animals and their high inter-individual variability, collection of biological samples and noninvasive imaging prior to irradiation should be maximized. After irradiation, evaluation of long term effects should be as comprehensive as possible, in order to allow discovery of unanticipated patterns of disease.

Acknowledgements

This work was supported by the Centers for Medical Countermeasures against Radiation Consortium, NIH grant U19-AI67798 awarded to Nelson Chao, Duke University (JMC: primate core leader, Wake Forest School of Medicine).

References

Allen RG, Brown FA, Logie LC, Rovner DR, Wilson SG Jr, Zellmer RW. Acute effects of gamma radiation in primates. Radiat Res. 1960 May;12:532-59. PubMed PMID: 13792780.

Bansode FW, Chowdhury SR, Dhar JD. Seasonal changes in the seminiferous epithelium of rhesus and bonnet monkeys. J Med Primatol. 2003 Jun;32(3):170-7. PubMed PMID: 12823627.

Broerse JJ, Bartstra RW, van Bekkum DW, van der Hage MH, Zurcher C, van Zwieten MJ, Hollander CF. The carcinogenic risk of high dose total body irradiation in non-human primates. Radiother Oncol. 2000 Mar;54(3):247-53. PubMed PMID: 10738083.

Caveness WF. Pathology of radiation damage to the normal brain of the monkey. Natl Cancer Inst Monogr. 1977 Dec;46:57-76. PubMed PMID: 418344.

Cline JM, Dugan GO, Andrews RN, Kavanagh K, Bourland D, Hanbury D, Peiffer A, Register T, Debo R, Caudell D, Chao N. Multisystemic late effects of radiation exposure in nonhuman primates. Abstract/Platform Presentation. Radiation Research Society Annual Meeting, 2016.

Cupp CJ, Uemura E. Body and organ weights in relation to age and sex in *Macaca mulatta*. J Med Primatol. 1981;10(2-3):110-23.

DeBo RJ, Lees CJ, Dugan GO, Caudell DL, Michalson KT, Hanbury DB, Kavanagh K, Cline JM, Register TC. Late Effects of Total-Body Gamma Irradiation on Cardiac Structure and Function in Male Rhesus Macaques. Radiat Res. 2016 Jul;186(1):55-64. doi: 10.1667/RR14357.1. PubMed PMID: 27333082; PubMed Central PMCID: PMC5068576.

Du Y, Fan TY, Tan Y, Xiong Z, Wang Z. Seasonal changes in the reproductive physiology of female rhesus macaques (Macaca mulatta). J Am Assoc Lab Anim Sci. 2010 May;49(3):289-93. PubMed PMID: 20587158; PubMed Central PMCID: PMC2877299.

Garofalo M, Bennett A, Farese AM, Harper J, Ward A, Taylor-Howell C, Cui W, Gibbs A, Lasio G, Jackson W 3rd, MacVittie TJ. The delayed pulmonary syndrome following acute high-dose irradiation: a rhesus macaque model. Health Phys. 2014 Jan;106(1):56-72. PubMed PMID: 24276550.

Gulani J, Koch A, Chappell MG, Christensen CL, Facemire P, Singh VK, Ossetrova NI, Srinivasan V, Holt RK. Cercopithecine Herpesvirus 9 (Simian Varicella Virus) Infection after Total-Body Irradiation in a Rhesus Macaque (Macaca mulatta). Comp Med. 2016 Apr;66(2):150-3. PubMed PMID: 27053570; PubMed Central PMCID: PMC4825965.

Hanbury DB, Robbins ME, Bourland JD, Wheeler KT, Peiffer AM, Mitchell EL, Daunais JB, Deadwyler SA, Cline JM. Pathology of fractionated whole-brain irradiation in rhesus monkeys (Macaca mulatta). Radiat Res. 2015 Mar;183(3):367-74. doi: 10.1667/RR13898.1. PubMed PMID: 25688996; PubMed Central PMCID: PMC4467778.

Hukkanen RR, Gillen M, Grant R, Liggitt HD, Kiem HP, Kelley ST. Simian varicella virus in pigtailed macaques (Macaca nemestrina): clinical, pathologic, and virologic features. Comp Med. 2009 Oct;59(5):482-7. PubMed PMID: 19887033; PubMed Central PMCID: PMC2771606.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 75 (2000). Ionizing Radiation, Part 1: X- and Gamma (γ)-Radiation, and Neutrons. Accessed 1.22.17 at http://monographs.iarc.fr/ENG/Monographs/vol75/

Jones JW, Bennett A, Carter CL, Tudor G, Hankey KG, Farese AM, Booth C, MacVittie TJ, Kane MA. Citrulline as a Biomarker in the Non-human Primate Total- and Partial-body Irradiation Models: Correlation of Circulating Citrulline to Acute and Prolonged Gastrointestinal Injury. Health Phys. 2015 Nov;109(5):440-51. doi: 10.1097/HP.0000000000000347. PubMed PMID: 26425904; PubMed Central PMCID: PMC4593331.

Kavanagh K, Dendinger MD, Davis AT, Register TC, DeBo R, Dugan G, Cline JM. Type 2 Diabetes is a Delayed Late Effect of Whole-Body Irradiation in Nonhuman Primates. Radiat Res. 2015 Apr;183(4):398-406. doi: 10.1667/RR13916.1. PubMed PMID: 25811716; PubMed Central PMCID: PMC4438751.

Kent SP, Pickering JE. Neoplasms in monkeys (Macaca mulatta): spontaneous and irradiation induced. Cancer. 1958 Jan-Feb;11(1):138-47. PubMed PMID: 13500309.

Kolappaswamy K, Mahalingam R, Traina-Dorge V, Shipley ST, Gilden DH, Kleinschmidt-Demasters BK, McLeod CG Jr, Hungerford LL, DeTolla LJ. Disseminated simian varicella virus infection in an irradiated rhesus macaque (Macaca mulatta). J Virol. 2007 Jan;81(1):411-5. PubMed PMID: 17079326; PubMed Central PMCID: PMC1797240.

Kolappaswamy K, Shipley ST, Tatarov II, DeTolla LJ. Methicillin-resistant Staphylococcus non-aureus infection in an irradiated rhesus macaque (Macaca mulatta). J Am Assoc Lab Anim Sci. 2008

Landauer MR, Srinivasan V, Seed TM. Genistein treatment protects mice from ionizing radiation injury. J Appl Toxicol. 2003 Nov-Dec;23(6):379-85. PubMed PMID: 14635262.

Lonser RR, Walbridge S, Vortmeyer AO, Pack SD, Nguyen TT, Gogate N, Olson JJ, Akbasak A, Bobo RH, Goffman T, Zhuang Z, Oldfield EH. Induction of glioblastoma multiforme in nonhuman primates after therapeutic doses of fractionated whole-brain radiation therapy. J Neurosurg. 2002 Dec;97(6):1378-89. PubMed PMID: 12507137.

MacVittie TJ, Bennett A, Booth C, Garofalo M, Tudor G, Ward A, Shea-Donohue T, Gelfond D, McFarland E, Jackson W 3rd, Lu W, Farese AM. The prolonged gastrointestinal syndrome in rhesus macaques: the relationship between gastrointestinal, hematopoietic, and delayed multi-organ sequelae following acute, potentially lethal, partial-body irradiation. Health Phys. 2012 Oct;103(4):427-53. doi: 10.1097/HP.0b013e318266eb4c. PubMed PMID: 22929471; PubMed Central PMCID: PMC4140097.

Marr-Belvin AK, Carville AK, Fahey MA, Boisvert K, Klumpp SA, Ohashi M, Wang F, O'Neil SP, Westmoreland SV. Rhesus lymphocryptovirus type 1-associated B-cell nasal lymphoma in SIV-infected rhesus macaques. Vet Pathol. 2008 Nov;45(6):914-21. doi: 10.1354/vp.45-6-914. PubMed PMID: 18984796; PubMed Central PMCID: PMC2735115.

Miller, AD. Neoplastic and Proliferative Disorders. In: Non-human Primates in Biomedical Research, v. 2, 2nd edition (2012), pg. 325-355

Moghaddam A, Rosenzweig M, Lee-Parritz D, Annis B, Johnson RP, Wang F. An animal model for acute and persistent Epstein-Barr virus infection. Science. 1997 Jun 27;276(5321):2030-3. PubMed PMID: 9197263.

Morton WR, Agy MB, Capuano SV, Grant RF. Specific pathogen-free macaques: definition, history, and current production. ILAR J. 2008;49(2):137-44. Review. PubMed PMID: 18323576.

Philipp MT, Doyle LA, Martin DS, Plauché GB, Phillippi-Falkenstein KM, Bohm RP Jr. A rhesus macaque model of Streptococcus pneumoniae carriage. J Med Primatol. 2012 Feb;41(1):60-6. doi: 10.1111/j.1600-0684.2011.00512.x. PubMed PMID: 21967372; PubMed Central PMCID: PMC3260395.

Price RE, Langford LA, Jackson EF, Stephens LC, Tinkey PT, Ang KK. Radiation-induced morphologic changes in the rhesus monkey (Macaca mulatta) brain. J Med Primatol. 2001 Apr;30(2):81-7. PubMed PMID: 11491408.

Rhesus Macaque Genome Sequencing and Analysis Consortium., Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, Mardis ER, Remington KA, Strausberg RL, Venter JC, Wilson RK, Batzer MA, Bustamante CD, Eichler EE, Hahn MW, Hardison RC, Makova KD, Miller W, Milosavljevic A, Palermo RE, Siepel A, Sikela JM, Attaway T, Bell S, Bernard KE, Buhay CJ, Chandrabose MN, Dao M, Davis C, Delehaunty KD, Ding Y, Dinh HH, Dugan-Rocha S, Fulton LA, Gabisi RA, Garner TT, Godfrey J, Hawes AC, Hernandez J, Hines S, Holder M, Hume J, Jhangiani SN, Joshi V, Khan ZM, Kirkness EF, Cree A, Fowler RG, Lee S, Lewis LR, Li Z, Liu YS, Moore SM, Muzny D, Nazareth LV, Ngo DN, Okwuonu GO, Pai G, Parker D, Paul HA, Pfannkoch C, Pohl CS, Rogers YH, Ruiz SJ, Sabo A, Santibanez J, Schneider BW, Smith SM, Sodergren E, Svatek AF, Utterback TR, Vattathil S, Warren W, White CS, Chinwalla AT, Feng Y, Halpern AL, Hillier LW, Huang X, Minx P, Nelson JO, Pepin KH, Qin X, Sutton GG, Venter E, Walenz BP, Wallis JW, Worley KC, Yang SP, Jones SM, Marra MA, Rocchi M, Schein JE, Baertsch R, Clarke L, Csürös M, Glasscock J, Harris RA, Havlak P, Jackson AR, Jiang H, Liu Y, Messina DN, Shen Y, Song HX, Wylie T, Zhang L, Birney E, Han K, Konkel MK, Lee J, Smit AF, Ullmer B, Wang H, Xing J, Burhans R, Cheng Z, Karro JE, Ma J, Raney B, She X, Cox MJ, Demuth JP, Dumas LJ, Han SG, Hopkins J, Karimpour-Fard A, Kim YH, Pollack JR, Vinar T, Addo-Quaye C, Degenhardt J, Denby A, Hubisz MJ, Indap A, Kosiol C, Lahn BT, Lawson HA, Marklein A, Nielsen R, Vallender EJ, Clark AG, Ferguson B, Hernandez RD, Hirani K, Kehrer-Sawatzki H, Kolb J, Patil S, Pu LL, Ren Y, Smith DG, Wheeler DA, Schenck I, Ball EV, Chen R, Cooper DN, Giardine B, Hsu F, Kent WJ, Lesk A, Nelson DL, O'brien WE, Prüfer K, Stenson PD, Wallace JC, Ke H, Liu XM, Wang P, Xiang AP, Yang F, Barber GP, Haussler D, Karolchik D, Kern AD, Kuhn RM, Smith KE, Zwieg AS. Evolutionary and biomedical insights from the rhesus macaque genome. Science. 2007 Apr 13;316(5822):222-34. PubMed PMID: 17431167.

Rivailler P, Carville A, Kaur A, Rao P, Quink C, Kutok JL, Westmoreland S, Klumpp S, Simon M, Aster JC, Wang F. Experimental rhesus lymphocryptovirus infection in immunosuppressed macaques: an animal model for Epstein-Barr virus pathogenesis in the immunosuppressed host. Blood. 2004 Sep 1;104(5):1482-9. PubMed PMID: 15150077.

Simmons HA, Mattison JA. The incidence of spontaneous neoplasia in two populations of captive rhesus macaques (Macaca mulatta). Antioxid Redox Signal. 2011 Jan 15;14(2):221-7.

Summers L, Clingerman KJ, Yang X. Validation of a body condition scoring system in rhesus macaques (Macaca mulatta): assessment of body composition by using dual-energy X-ray absorptiometry. J Am Assoc Lab Anim Sci. 2012 Jan;51(1):88-93. PubMed PMID: 22330874; PubMed Central PMCID: PMC3276972.

Thigpen JE, Setchell KD, Saunders HE, Haseman JK, Grant MG, Forsythe DB. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J. 2004;45(4):401-16. Review. PubMed PMID: 15454679.

Wilson GM. An Illustrated Guide to Primate Necropsy. Masters Thesis, Southwester Graduate School of Biomedical Science, The University of Texas Health Science Center at Dallas. 1985. Accessed via www.cldavis.org/PDFs/NHP_Necropsy_Guide_Wilson.pdf *on 1/2/17*

Wood CE, Usborne AL, Starost MF, Tarara RP, Hill LR, Wilkinson LM, Geisinger KR, Feiste EA, Cline JM. Hyperplastic and neoplastic lesions of the mammary gland in macaques. Vet Pathol 2006;43:471-483. PMID: 16846989

Wood CE, Borgerink H, Register TC, Scott L, Cline JM. Cervical and vaginal epithelial neoplasms in cynomolgus monkeys. Vet Pathol 2004;41(2):108-115. PMID: 15017023

Wood CE, Chen Z, Cline JM, Miller BE, Burk RD. Characterization and experimental transmission of an oncogenic papillomavirus in female macaques. J Virol 2007;81(12):6339-6345. PMCID: PMC1900122