Molecular and cellular profiling of acute responses to total body radiation exposure in ovariectomized female cynomolgus macaques

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Abstract

Purpose—The threat of radiation exposure requires a mechanistic understanding of radiation-induced immune injury and recovery. The study objective was to evaluate responses to ionizing radiation in ovariectomized (surgically post-menopausal) female cynomolgus macaques.

Materials and methods—Animals received a single total-body irradiation (TBI) exposure at doses of 0, 2 or 5 Gy with scheduled necropsies at 5 days, 8 weeks and 24 weeks post-exposure. Blood and lymphoid tissues were evaluated for morphologic, cellular, and molecular responses.

Results—Irradiated animals developed symptoms of acute hematopoietic syndrome, and reductions in thymus weight, thymopoiesis, and bone marrow cellularity. Acute, transient increases in plasma monocyte chemoattractant protein 1 (MCP-1) were observed in 5 Gy animals along with dose-dependent alterations in messenger ribonucleic acid (mRNA) signatures in thymus, spleen, and lymph node. Expression of T cell markers was lower in thymus and spleen,

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while expression of macrophage marker CD68 (cluster of differentiation 68) was relatively elevated in lymphoid tissues from irradiated animals.

Conclusions—Ovariectomized female macaques exposed to moderate doses of radiation experienced increased morbidity, including acute, dose-dependent alterations in systemic and tissue-specific biomarkers, and increased macrophage/T cell ratios. The effects on mortality exceeded expectations based on previous studies in males, warranting further investigation.

Keywords

Radiation; non-human primate; immunology; molecular profiling; total body irradiation; acute effects; macrophage; cytokines

Introduction

Threat of accidental or malicious exposure of the public or the military to ionizing radiation necessitates development of effective pre- and post-exposure countermeasures. Development of radiation countermeasures also provides potentially valuable strategies for avoidance of normal tissue damage in patients given radiation therapy. A critical component of ionizing radiation damage is loss of protective immune function caused by depletion of nascent and developing immune cells. Immune system compromise in turn can result in significant morbidity and mortality. Detailed examination of the complex processes underlying response to, and recovery from, acute ionizing radiation exposure requires use of small and large animal models.

Rodent models are widely used for assessment of immune function; however, they differ significantly from humans in several characteristics, such as functional markers of natural killer and T cells, proteins involved in antigen processing and presentation and genomic responses to inflammatory diseases (Seok et al. 2013). Old world monkeys such as the cynomolgus macaque have close evolutionary relationships to humans and in some contexts are more appropriate models for evaluating immune system function and host response to infectious diseases, having similar susceptibility to common pathogens such as measles and influenza, and immunosuppressive lentiviruses (Palermo et al. 2013). The utility of the rhesus macaque non-human primate (NHP) model has been well-established to study immune system homeostasis and function, general physiology, and a wide range of pathologic diseases and conditions (Messaoudi et al. 2011) and its utility in radiation countermeasure research is clear (Chen et al. 2010, Robbins et al. 2011, Farese et al. 2012, MacVittie et al. 2012).

The purpose of the present study was to define cellular and molecular changes occurring in critical central and peripheral immune compartments in response to moderate levels of acute total body ionizing radiation exposure in an alternative species of macaque; the cynomolgus macaque (*Macaca fascicularis*). The cynomolgus macaque is a well-established NHP model of health and disease which provides some advantages over rhesus in terms of availability and size.

Specifically we determined the effects of radiation and recovery time on general health and clinico- pathologic indices; plasma cytokines; and expression of markers of individual cell types in immune compartments (thymus, spleen, and lymph node). Time- and dose-dependent acute systemic responses to ionizing radiation exposure, as well as tissue specific biomarker responses, were observed. The results provide a multi-dimensional profile of responses of female cynomolgus monkeys to graded doses of radiation that will help inform future studies utilizing cynomolgus macaques investigating the effects of radiation exposure.

Methods

Subjects and study design

Fifteen ovariectomized adult female cynomolgus monkeys (*Macaca fascicularis*) weighing a median of 3.03 kg (2.56–4.59 kg) were obtained from the Institut Pertanian Bogor and had completed prior studies of dietary protein (from soy and milk sources) with varying soy isoflavone content (Wood et al. 2006, Adams et al. 2008) at the Wake Forest School of Medicine Center for Comparative Medicine Research. These well-characterized, 'late-middle aged' female monkeys with an estimated median age of 20 years (15–24 years) were screened for exclusion of simian retroviruses and were in excellent health prior to the study. All experimental procedures were in compliance with the Wake Forest University Institutional Animal Care and Use Committee and the Guide for Care and Use of Laboratory Animals. Animals were screened prior to irradiation by clinical examination, complete blood count, and serum chemistry panels. Monkeys were randomized to three groups (0, 2 or 5 Gy), with timed necropsy evaluations planned at 5, 60 and 180 days post-irradiation. Complete animal demographic information can be found in Table I.

Diet

Following a three-month period of consumption of a laboratory chow diet (Purina LabDiet® Monkey Diet 5037, Richmond, IN, USA), animals were transitioned to a diet designed to mimic a typical Western diet a minimum of two weeks prior to irradiation and maintained on this diet for the duration of the study. This diet was produced in house and contained 34% of calories as fat, 18% of calories as protein, and 48% of calories as carbohydrates, with a moderately atherogenic cholesterol content of 0.20 mg/Cal. Protein was derived solely from animal sources (casein, lactalbumin). Of the fat, fatty acid composition was 40% saturated, 34% monounsaturated and 26% polyunsaturated. Monkeys were supplemented with fresh fruits and vegetables and water *ad libitum*.

Irradiation

Monkeys were anesthetized with ketamine (10 mg/kg) (Vedco Inc., St Joseph, MO, USA) and irradiated to single doses of 0, 2 or 5 Gy to the mid-plane using two opposed lateral fields of 6 MV X-rays at a dose rate of 60 cGy/min from a Varian 2100C linear accelerator (Varian Medical Systems, Inc., Palo Alto, CA, USA). The radiation source to skin distance was 120 cm.

Clinical monitoring and husbandry

On the day of irradiation, animals were given parenteral prophylactic antibiotic treatment consisting of enrofloxacin (5 mg/kg) (Bayer HealthCare LLC, Shawnee Mission, KS, USA) and ceftazidime (30 mg/kg) (Patterson Veterinary, Devens, MA, USA), continuing until the white blood cell count returned to normal range ($6.1–12.5 \times 10^3$ cells/ul) or until clinical illness (if present) was resolved. Control animals were maintained on antibiotics for the duration of the study. Clinical assessments were performed daily to monitor for signs of radiation-induced illness including vomiting, diarrhea, weight loss, hemorrhage, and alopecia. Signs of hematologic failure or infection were monitored using the methods of Uckun et al. (1997) as modified from the Children's Cancer Group Clinical Toxicity Criteria. Supportive care was provided when clinically necessary and included, parenteral fluids and supplemental nutrition. Blood transfusions were not given. In the event that animals became moribund, or reached the level of Grade 4 toxicity, as previously used in primate trials at Wake Forest (Hotchkiss et al. 1999, Cohen et al. 2004), they were humanely euthanized by intravenous pentobarbital overdose.

Hematology

Complete blood counts (hematocrit, red blood cells, total and differential leukocyte counts, hemoglobin, platelets) and chemistry panels (serum blood urea nitrogen, creatinine, total protein, albumin, bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, potassium, sodium, chloride, total CO_2) were collected and analyzed three times weekly for a period of one month following irradiation. Analyses were conducted in-house with a Heska Hematrue analyzer (Heska, Loveland, CO, USA) and by an external commercial veterinary laboratory (Antech Diagnostics, South Haven, MS, USA).

Necropsy/histopathology

All animals were humanely euthanized for collection and evaluation of major organs/tissues, with a particular focus on thymus, spleen, lymph nodes, and bone marrow. Thymus and spleen were collected and portions were snap frozen and stored at $-80°C$, minced and incubated in RNA$_{later}$® (Qiagen, Austin, TX, USA) until freezing, or fixed with 4% paraformaldehyde followed by paraffin embedding and sectioning for histopathology. Similarly, small pieces of axillary, inguinal and submandibular lymph node tissues were snap frozen for analysis as described below.

Molecular assessment of thymic output

T-cell receptor (TCR) gene rearrangement and thymopoiesis were determined by quantification of signal joint TCR excision circles (sjTREC) (Lynch and Sempowski 2013). Recombinant sjTREC (NHP) plasmid control was generously provided by Dr. Danny Douek (VRC-NIAID).

Molecular characterization of lymphoid tissue cell composition

Quantitative real-time polymerase chain reaction (RTPCR) was used to evaluate the inflammatory cell content of thymus, spleen, and lymph node using previously described

methods (Sophonsritsuk et al. 2013; Walker et al. 2008a, 2008b). Levels of mRNA transcripts for a specific marker associated with macrophages cluster of differentiation 68 (CD68), and four markers of T cells T-cell receptor beta, cluster of differentiation 3, 4 and 8 (TCRB, CD3, CD4, CD8) were measured in order to define the cellular composition of the tissue. Total ribosomal nucleic acid (RNA) was purified by homogenization of tissue preserved in RNA_{later} using TRI Reagent (Molecular Research Center, Inc.; Cincinnati, OH, USA) as described by Chomczynski (1993). RNA content and purity was determined at 260 nm and using A260/A280 ratios obtained on a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA quality was assessed by intensity of 28S and 18S ribosomal RNA bands on an Agilent Microanalyzer. Quantitative Taqman® RT-PCR assays employing cynomolgus-specific primers and Fam-labeled Taqman® MGB probes were used to quantitate mRNA steady-state levels (Sophonsritsuk et al., 2013; Walker et al., 2008a, 2008b). Aliquots of purified total RNA (~ 1–10 ug RNA) were reverse transcribed using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) in combination with random hexamers to generate cDNA from which real time RT-PCR quantitative analyses were performed. Aliquots of the cDNA archive were amplified with target-specific primers along with Taqman® probes (for targets described above). Real time quantitative RT-PCR was carried out on an Applied Biosystems ABI PRISM® 7000 Sequence Detection System. PCR was performed in separate wells for each target using a Taqman® Universal Master Mix and associated protocols. For each sample, the threshold cycle (Cts) for the gene of interest and the selected housekeeping gene(s) were determined. The ΔCt [Ct (target gene)-Ct (housekeeping gene(s))] was calculated to provide a measure of relative gene expression as noted above. Target gene expression was normalized to the geometric mean of three control genes [β-actin, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and ribosomal protein, large, P0 (RPLP0)] in each sample.

Acute inflammatory response to radiation

Cytokine analyses were performed on plasma samples collected on days 0, 1, 2, 4 and 6 of the first week, and every other day thereafter for the following 5 weeks, and then at weekly or monthly intervals thereafter. Analyses were carried out using a Luminex bead-based monkey 10-plex assay from Biosource/Life Technologies (Grand Island, NY, USA) for measurement of Interferon-γ, tumor necrosis factor-α, interleukins 2, 4, 8, 10, monocyte chemoattractant protein-1, macrophage inflammatory protein 1 alpha and beta, and Regulated on Activation, Normal T Cell Expressed and Secreted (IFNγ, TNFα, IL-2, IL-4, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, and RANTES). Values at or below the low limit of detection (LLOD) were reported at one-half the values of the LLOD.

Statistics

Statistically, the overall experiment was considered a pilot study with multiple time-points and a small number of subjects in individual cells, thus not lending itself well to traditional statistical approaches. Descriptive statistics were generated for all outcomes across the duration of the study. As noted above, necropsies were conducted at different time-points following irradiation. Some tissue outcomes (e.g., sjTREC analysis, organ weight, cell marker mRNA levels) showed dose-dependent effects which appeared to be only minimally affected by time since irradiation and were pooled across time-points and analyzed by

Kruskal-Wallis non-parametric analysis of variance (ANOVA). Spear-man rho and Kendall's tau correlations were determined to estimate trends and dose response relationships with some variables. A *p* value of < 0.05 was considered significant. As a descriptive analysis, no adjustments for multiplicity were made. Analyses were conducted using JMP Statistical Discovery Software (Version 3.2.2, SAS Institute, Inc., Cary, NC, USA) or Statistica (Statsoft Inc, Tulsa, OK, USA).

Results

Clinical observations

Nausea or vomiting immediately after irradiation was seen in 0/3 controls, 1/6 animals given the 2 Gy dose, and 2/6 animals given the 5 Gy dose. Weight loss (5–15% of pre- irradiation body weight) was seen in 0/3 controls, 2/6 animals given 2 Gy, and 6/6 animals given 5 Gy. Early humane euthanasia was elected in 2/6 animals in the 2 Gy group (at 20 days post-irradiation) and 3/6 animals in the 5 Gy dose group (at 15, 16 and 17 days post-irradiation). due to severe clinical illness. Hemorrhage was seen in most irradiated animals (skin/ subcutis, gingiva, lung, epicardium, skeletal muscle); this was attributed to thrombocytopenia.

Clinical pathology findings

Dose-dependent decreases were seen in platelet counts, white blood cell counts, and red blood cell (RBC) counts. Platelets declined on day 2, reaching a nadir on average of 22,250 cells/ul approximately 14–23 days after irradiation with recovery to an average of > 300,000 cells/ul approximately 30–45 days after irradiation in survivors (Figure 1A). Neutro-phil counts (Figure 1B) increased acutely at day 2, and then declined to an average nadir of ~ 990 cells/ul approximately 6–14 days after irradiation. Lymphocyte counts declined to ~ 1600 cells/ul by day 2, reaching a nadir 6–10 days post irradiation which persisted for at least 6 months (Figure 1C). RBC counts in both irradiated groups declined (Figure 1D). RBC levels for the 5 Gy dose group failed to recover, while the monkeys receiving the 2 Gy dose exhibited a gradual increase in RBC count approximately 30 days after radiation, which eventually returned to non-irradiated control levels (0 Gy). Mortality increased with radiation dose and overall survival time post irradiation decreased with dose (Table I).

Impact of ionizing radiation on lymphoid and hematopoietic tissues

Severe hematopoietic cell depletion was observed across all lineages in the bone marrow of animals that received 2 or 5 Gy radiation (Figure 2A–C). Lymphocyte depletion in the spleen (and lymph node, data not shown) was noted at necropsy in all irradiated animals (Figure 2D–F). One animal in the 5 Gy group developed cellulitis and had bacterial colonies in the lungs, consistent with sepsis.

Significantly lower thymic weight was observed in animals receiving radiation compared to controls (both $p < 0.04$, control vs. 2 Gy $p = 0.04$, control vs. 5 Gy $p = 0.02$, Figure 3A), and there was an inverse relationship between thymic weight and radiation dose (Spearman rho $= -0.546$, $p = 0.05$). Thirteen thymic samples yielded good quality genomic DNA capable of generating a thymic sjTREC result; due to the small group size, data from specific doses

were grouped independent of time to show the overall impact of irradiation on thymopoiesis. The data show sjTREC, or TCR gene rearrangement, tended be lower with 2 and 5 Gy irradiation ($p = 0.053$) (Figure 3B), suggesting diminished T cell development, a finding consistent with the histologic demonstration of marked cortical and medullary atrophy in thymic tissues from these animals (Figure 3C).

Tissue expression levels of key mRNA transcripts evaluated by qRT-PCR indicated radiation-induced shifts in the relative cell populations present in lymphoid tissues. Expression of T cell markers was reduced with radiation exposure; CD3 mRNA was significantly reduced in thymus ($p = 0.006$) and lymph node ($p < 0.004$) but not spleen ($p > 0.1$) (Figure 4), while CD4 mRNA was significantly reduced in thymus ($p = 0.001$, data not shown). CD68 expression tended to be elevated in the irradiated thymus ($p = 0.07$) lymph node ($p = 0.09$), and spleen ($p = 0.11$). Radiation exposure was inversely correlated with expression of CD3 in lymph node (tau $= -0.41$, $p = 0.054$) and thymus (tau $= -0.53$, $p = 0.014$), with CD4 in thymus (tau $= -0.598$, $p = 0.005$), and positively correlated with CD68 expression in lymph node (tau $= 0.506$, $p = 0.018$) and thymus (tau $= 0.55$, $p = 0.01$). Dose-dependent increases in expression ratios of macrophage/T cell markers (CD68/CD3) was observed in Spleen and Lymph Node. An increase in expression of macrophage/T cell marker ratios was also observed in the thymus of animals exposed to 5 Gy (results not shown).

Acute cytokine response to radiation

The effects of total body irradiation on systemic levels of plasma cytokines IFNγ, TNFα, IL-2, IL-4, IL-10, MCP-1, MIP-1α, MIP-1β, RANTES and IL-8 were evaluated over time post irradiation. The most consistent radiation-related finding was an increase in plasma MCP-1 levels in the group receiving the 5 Gy dose (Figure 5). Changes over time in other cytokines were variable, and sometimes exhibited changes in controls which could be responses to frequent blood draws or other factors (see Supplementary Figure 1, available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1028597).

Discussion

Previous studies of the effects of ionizing radiation in the non-human primate have been primarily done in male animals, with most of the recent work performed in adolescent or young adult male rhesus monkeys (Farese et al. 2001, 2003, 2012, MacVittie et al. 2012, Dörr et al. 2014). The purpose of the present study was to evaluate responses to graded doses of total body radiation in the ovariectomized female cynomolgus monkey, a well-established model of post-menopausal female health. Our major long-term goal is to develop medical countermeasures that minimize the adverse health consequences of unintentional (accidental or terrorism-related) exposures to radiation in men and women across their lifespan. The present study lays some of the groundwork necessary for the development of an alternative and relatively more available animal model for evaluation of radiation mitigation strategies in post-menopausal women.

Cynomolgus macaques are an excellent model for the study of inflammation and cardiovascular disease (Register 2009), cancer risk (Cline and Wood 2009), and other

conditions, and may provide a useful addition to our non-human primate animal model portfolio for discovery and development of radiation countermeasures. The effects of radiation exposure on stem cells (Adler and Erbelding 1988), brain electrical activity (Legeza and Turlakov 1991), liver function (Yannam et al. 2014), clinical, cardiac, cortisol stress response (Darenskaia et al. 1992, 2001), and mitigating effects of gonadotropin hormone releasing hormone (GnRH) antagonism on the testes (Shetty et al. 2013) have been studied in this species. Additional studies focusing on immune tissues/organs in this species should facilitate identification of pathways to immune dysfunction and hypothesis generation leading to novel pharmacologic and/or nutritional countermeasures to prevent or treat adverse effects of radiation on at risk organ systems.

In this study ovariectomized female cynomolgus monkeys exposed to a single 2–5 Gy dose of total body ionizing radiation experienced reductions in thymus weight and thymopoiesis, loss of cortical thymocytes, depletion of bone marrow, and peripheral hemorrhage which was attributed to thrombocytopenia. Radiation dose-dependent decreases in platelet, white blood cell (WBC), and red blood cell (RBC) counts were also observed. Similar patterns of neutropenia, thrombocytopenia, along with transient increases in systemic inflammatory cytokines (e.g., IFNγ, MCP-1, and MIP-1), and subsequent recovery have been observed in male rhesus macaques exposed to total body irradiation doses in the range of 7–8.9 Gy, which is consistent with 30–90% lethality at 60 days (Farese et al. 2012).

Altered mRNA signatures in thymus, spleen, and lymph node were observed in our cohort of irradiated cynomolgus female animals. CD3 (a marker of T cells) demonstrated tissue specific responses; exhibiting decreased expression with increasing radiation dose in thymus and lymph node, but not spleen; while expression of the macrophage marker CD68 increased in a dose-dependent manner relative to T cell-specific marker expression. These results suggest exposure to ionizing radiation may induce acute, dose-dependent alterations in systemic and tissue-specific biomarker responses with increases in macrophage/T cell ratios in lymphoid tissues. Histologic evaluations of these tissues suggested an overall reduction in the number of lymphocytes present, so the observed increase in macrophage/T cell marker ratios in these lymphoid tissues may reflect persistence of resident macrophages.

This study was designed to provide pilot data regarding the effects of sub-lethal and theoretically low to moderate single doses of total body irradiation at 2 and 5 Gy in ovariectomized adult female cynomolgus monkeys. Radiation dose levels of 2 and 5 Gy were selected largely based upon known responses of pubertal and adult male rhesus monkeys to total body irradiation (Farese et al. 2001, 2003, 2012, Stickney et al. 2006, Hérodin et al. 2007, Burdelya et al. 2008). Ovariectomized female cynomolgus monkeys were more radiosensitive than anticipated, as 2/6 animals receiving the 2 Gy dose and 3/6 of the animals receiving the 5 Gy dose experienced significant health problems and were euthanized ahead of schedule on the basis of our predefined health criteria, indicating that future non-lethal studies utilizing this model of radiation exposure should use total body doses lower than 5 Gy. Estimates of exposures from Nagasaki data suggest that the lethal dose in humans at 60 days for 50% of those exposed (LD_{50}) is approximately 4.1 Gy (Anno et al. 2003).

The radiosensitivity differences observed here could be due to species, sex, and/or menopausal status. Estrogen has been shown to modulate immune responses is some systems through estrogen receptor interactions with the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (McKay and Cidlowski 1999, Kalaitzidis and Gilmore 2005, De Bosscher et al. 2006), and the lack of endogenous estrogen in these ovariectomized animals may have influenced responses to radiation. Influences of sex or endogenous sex hormones on the responses to radiation have not been well-studied, especially in non-human primate models, although there is evidence in the literature of an effect of sex. In rats exposed to total body radiation, males appeared to exhibit increased survival over females in late stages after exposure (Peters et al. 1981). Markedly dissimilar responses to acute and chronic radiation exposure in the transcriptional profiles of skeletal muscle of male and female mice have also been reported (Kovalchuk et al. 2004). In rhesus monkeys, there appeared to be no difference between males and females in susceptibility to radiation-induced cataract formation (Sonneveld et al. 1979). Women are also at greater risk for developing pneumonitis following radiation to the lung (Baker et al. 2013). Female sex has also recently been shown to be a strong predictor of acute (odds ratio [OR]: 1.72) and late (OR: 3.96) radio toxicities (Meyer et al. 2012). Furthermore, a recent study showed that women had two-fold greater risk for the development of cancer following low-dose ionizing radiation for cardiac imaging than men (Lawler et al. 2013). Women also tend to be at greater risk for future cancer development following total body irradiation. According to the 14th report on mortality in the Life Span Study cohort of atomic bomb survivors, females had excess relative risks (ERR) twice as high as males for total deaths and all solid cancer deaths (Ozasa et al. 2012).

These findings suggest that more investigation is needed to derive definitive conclusions as to the effects of male and female sex, as well as reproductive stage (premenopausal versus post-menopausal), on molecular and cellular effects of total body irradiation (TBI). Future studies addressing these questions, as well as aim how the immune system reconstitutes/rebalances after radiation exposure are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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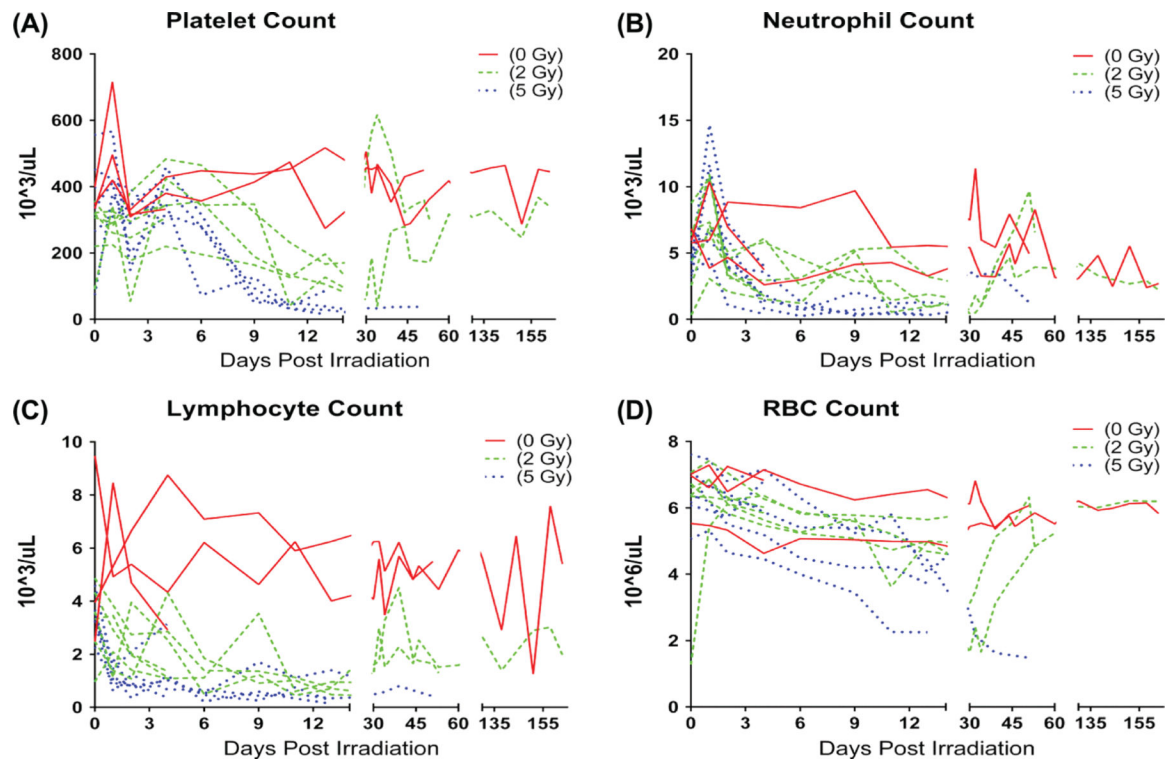**Figure 1.**
Effects of 2 and 5 Gy TBI on circulating blood components: (A) Platelets, (B) Neutrophils, (C) Lymphocytes, and (D) Erythrocytes. Single data points from individuals plotted across time. This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*
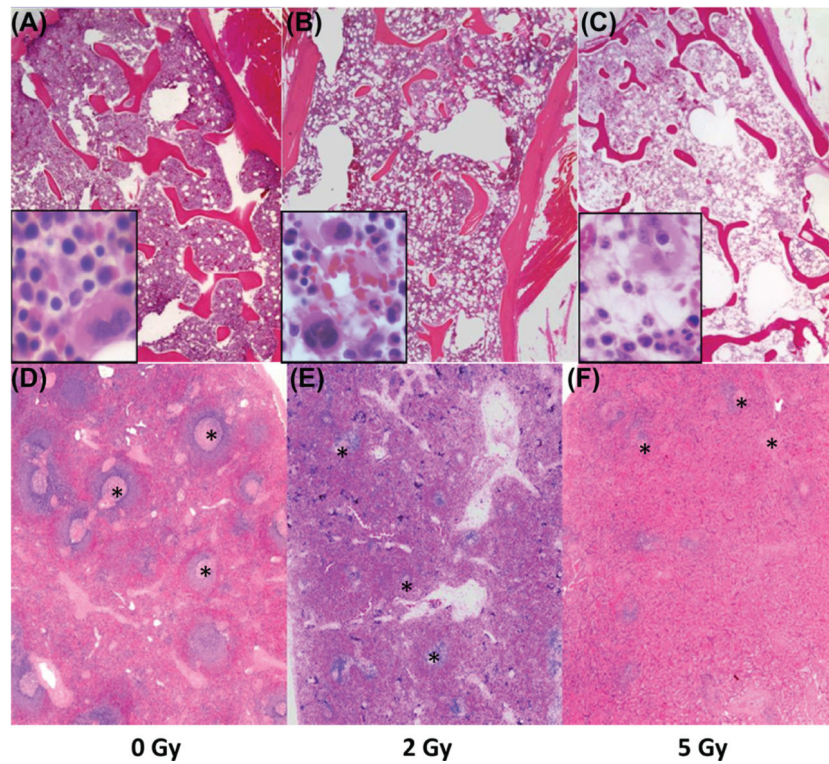
Figure 2.
Histologic sections of bone marrow (A–C) and spleen (D–F) from a control animal (0 Gy, A and D) and irradiated animals (2 Gy, B and E; and 5 Gy, C and F) five days post radiation exposure. The bone marrow from the irradiated animals was severely hypocellular with necrosis and loss of cells across all hematopoietic lineages. Note the dose-dependent marked necrosis and depletion of lymphoid follicles in the spleens of irradiated animals. Hematoxylin and eosin stain. Magnification 2×; inset magnification; 40×. Areas of splenic white pulp are marked with an (*). This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*
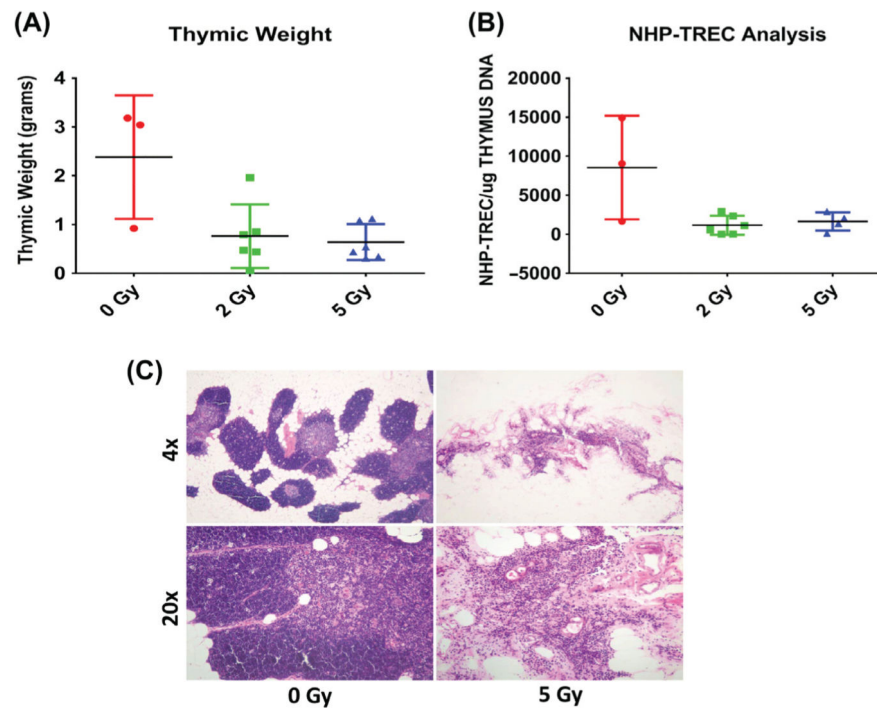
Figure 3.
Effects of 2 and 5 Gy TBI on thymus weight (A), thymic sjTREC levels, (B) and thymic histology (C). Data are mean ± SEM of *n* = 3–6 per group. Data from samples from each dose at different post-irradiation time-points were pooled for analysis due to the low number of observations and the lack of evidence for a time dependent trend. In the 0 and 5 Gy histologic sections note the marked loss of architecture (almost complete loss of cortical lymphocytes), with relative sparing of medullary epithelial elements, in the thymus from the animal treated with 5 Gy (right panels), compared to an un-irradiated control (left panels). Hematoxylin and eosin stain. Upper panels 4×, lower panels 20×. This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*
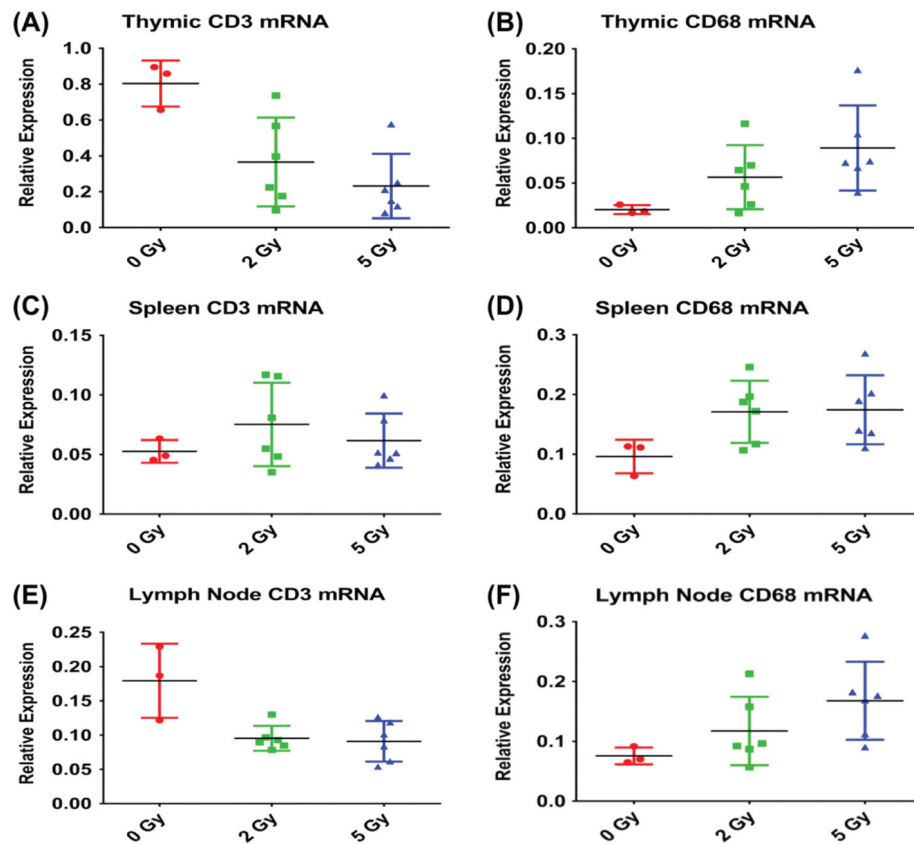
Figure 4.
Effects of 2 and 5 Gy TBI on levels of mRNA for T cell (CD3) and macrophage (CD68) markers as indices of tissue cell populations in lymph node, spleen, and thymus. Data from samples from each dose at different post-irradiation time-points were pooled for analysis due to the low number of observations and the lack of evidence for a time-dependent trend. Error bars indicate the standard error of the mean (SEM) for $n = 3$–6 per group. This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*
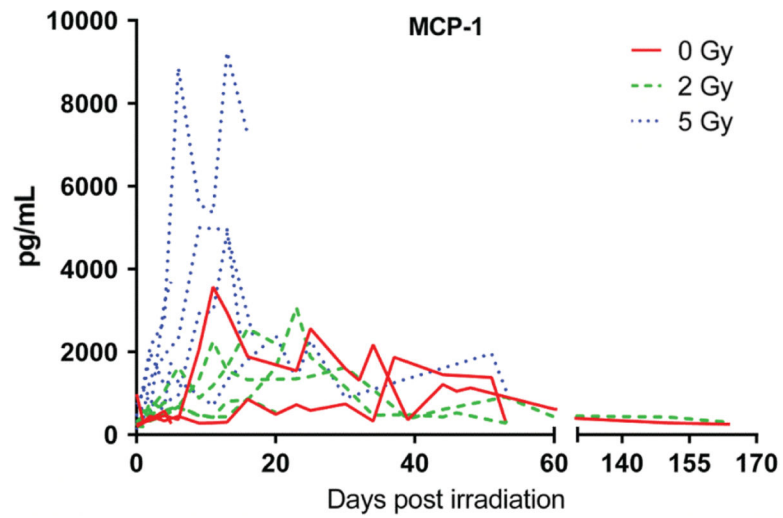
Figure 5.
Effects of 2, and 5 Gy TBI on plasma MCP-1. Single data points from individuals plotted across time. This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*

Table I

Complete animal demographic information of study to explore responses to ionizing radiation in ovariectomized female cynomolgus macaques.

ID	Radiation dose (Gy)	Days survived post irradiation	Death comments	Estimated age at death (yrs)	Baseline body weight (kg)	Body weight at death (kg)
A	0	–	Scheduled	20.2	2.9	3.0
B	0	–	Scheduled	18.9	4.0	3.5
C	0	–	Scheduled	19.7	2.9	2.8
D	2	5	Scheduled	21.7	4.7	4.6
E	2	5	Scheduled	15.2	2.9	2.6
F	2	20	Unexpected	21.8	2.9	2.8
G	2	20	Unexpected	23.7	3.5	3.0
H	2	53	Scheduled	20.8	3.6	3.3
I	2	165	Scheduled	20.2	3.4	3
J	5	5	Scheduled	22.7	2.9	2.8
K	5	5	Scheduled	18.7	3.1	3.0
L	5	15	Unexpected	20.8	3.1	3.1
M	5	16	Unexpected	19.8	3.3	3.3
N	5	17	Unexpected	20.8	3.9	3.8
O	5	53	Scheduled	14.9	4.6	4.0
Mean	**0**	**74**	**–**	**19.6**	**3.3**	**3.1**
Mean	**2**	**45**	**–**	**20.6**	**3.5**	**3.2**
Mean	**5**	**19**	**–**	**19.6**	**3.5**	**3.3**

Rows (A-O) are individual animal demographics. Mean rows are dose group means. Gy, Gray; Yrs, years; kg, kilograms.