Sex-Dependent Cytokine Responses to High-Dose Radiation Exposure in a Mouse Partial Body Irradiation Model

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Abstract

- **Background:** Cytokines are emerging biomarkers for radiation biodosimetry and injury assessment, yet the impact of biological sex on radiation-induced cytokine responses remains poorly defined. This study systematically evaluated sex differences in serum cytokine profiles following high-dose partial-body irradiation in mice.
- **Materials and Methods:** Male and female C57BL/6 mice (n=4/group) received 12–16 Gy partial-body irradiation with 5% bone marrow sparing (PBI/BM5). Serum was collected on Days 1, 3, and 7 post-irradiation along with naïve (0 Gy) controls. Seventy cytokines were measured. Baseline sex differences were assessed using independent t-tests with Benjamini–Hochberg False Discovery Rate (FDR) correction. Irradiated samples were normalized to sex-matched naïve baselines to isolate radiation-induced changes. PERMANOVA (9,999 permutations) quantified the variance in cytokine profiles attributable to sex. Radiation-responsive cytokines were defined by FDR q<0.05 and $|\log_2 FC|>1$ (≥2-fold change) across all three time points.
- **Results:** At baseline, 13 cytokines differed nominally between sexes (p<0.05), but Exodus-2/CCL21 remained significant after FDR correction. After normalization, PERMANOVA showed that sex explained ~20% of variance in radiation-induced cytokine profiles at all time points (p<0.0001). Five cytokines (GDF-15, FLT3L, IL-7, BAFF, MCP-3) were consistently altered in both sexes, defining a conserved core. Males showed sustained suppression of IFNγ, Granzyme B, and IL-11, with elevated IL-17F, while females showed sustained upregulation of Fractalkine/CX3CL1 and MCP-5. Radiation dose responses were minimal on Days 1–3 but emerged at Day 7, with 4 shared, 9 female-specific, and 2 male-specific cytokines.
- **Conclusions:** Sex is a major determinant of cytokine responses to high-dose radiation (~20% variance). Despite a shared core signature, males show reduced cytotoxic signaling, whereas females exhibit enhanced chemokine responses. Dose-dependent effects are most evident at later time points and are sex-specific, highlighting sex as a critical variable in radiation biology.

Introduction

- Radiological and nuclear mass-casualty events demand rapid, accurate biodosimetry to guide triage and medical countermeasure deployment (1,2).
- Circulating cytokines are promising radiation biomarkers, reflecting systemic immune activation, hematopoietic injury, and tissue damage following exposure (3-5).
- Most preclinical radiation studies use whole-body irradiation (WBI), which does not replicate realistic radiation exposure scenarios; partial-body irradiation with bone marrow sparing (PBI/BM5) more accurately models real-world exposures where shielding of residual bone marrow enables survival and recovery (6-8).
- Biological sex is a well-established modulator of immune function, with females generally mounting stronger innate and adaptive immune responses than males; however, the impact of sex on radiation-induced cytokine responses remains undefined (9-10).
- No study to date has systematically characterized sex-specific cytokine dynamics in a clinically relevant partial-body irradiation model across multiple doses and time points
- This study addresses this critical gap by profiling 70 serum cytokines in male and female C57BL/6 mice following PBI/BM5 at doses ranging from 12–16 Gy, at Days 1, 3, and 7 post-irradiation, to identify sex-specific radiation biomarkers and characterize dose-dependent cytokine responses.

Materials and Methods

- ***Mice and Animal Care:*** 10- to 14-week-old C57BL6 male and female mice (Jackson Lab) were used for the current study. Mice were randomized for each experimental group and housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facility at the Armed Forces Radiobiology Research Institute (AFRRI). The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC). Animal rooms were maintained at 20-26°C with 30-70% humidity on a 12 h light/dark cycle. Commercial rodent chow (Harlan Teklad Rodent Diet 2018) was available *ad libitum* as was acidified water (pH = 2.5-3.0) to control opportunistic infections.
- ***Irradiation:*** PBI/BM5 was carried out using an Elekta Infinity™ clinical linear accelerator (LINAC) at AFRRI to provide precise partial body irradiation. PBI was carried out at an estimated dose rate of 2.75 Gy/min. The mice were anesthetized during irradiation using ketamine+xylazine. For PBI/BM5, the mice were exposed to specified doses of radiation to the whole body except the hind limbs' fibulae, tibiae, and feet (which constituted about 5% of bone marrow). All animals were transferred back to their respective cages for recovery from anesthesia and general physical appearance were monitored. The day of irradiation was considered day 0.
- ***Tissue collection:*** On days 1, 3, and 7 after IR, mice were anesthetized for blood collection and then euthanized for collection of additional tissues.
- ***cytokine assay***: Circulating cytokine levels were analyzed using the Eve Technologies 68-plex cytokine assay, which quantifies a broad panel of inflammatory and immune-regulatory cytokines. Additionally, IL-18 and IL-18BP were measured using specific ELISAs.
- ***Statistical analysis***: Cytokine concentrations were $\log_2$-transformed prior to analysis; 5 cytokines falling outside the assay's linear detection range were excluded, leaving 65 cytokines for analysis. Baseline sex differences were assessed using independent samples t-tests with Benjamini–Hochberg (BH) FDR correction across 65 cytokines. Irradiated samples were normalized to sex-matched naïve means ($\log_2$FC vs. naïve) to isolate radiation-induced changes independent of baseline sex differences Multivariate cytokine profiles were compared between sexes using PERMANOVA (Euclidean distance, 9,999 permutations); homogeneity of dispersion was verified by PERMDISP. Radiation-responsive cytokines were identified by one-sample t-tests against zero with BH FDR correction, and defined as FDR q<0.05 and $|\log_2 FC|>1$ across all three post-irradiation time points. Sex differences in radiation-induced responses were assessed by independent t-tests with BH FDR correction at each time point. Dose-dependent responses were evaluated using Spearman rank correlation between cytokine $\log_2$FC and radiation dose (12–16 Gy), with BH FDR correction per group. All analyses were performed in Python (v3.12).

Fig. 1.showed the experimental design.

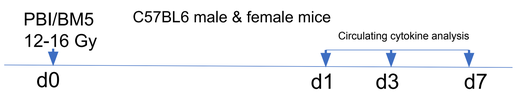

Fig. 1. Scheme of the study design. Male and female C57BL/6 mice were exposed to 12–16 Gy PBI/BM5 irradiation. Mice were euthanized on days 1, 3, and 7 post-irradiation. Circulating cytokine levels were measured and analyzed to assess the effects of radiation dose and sex.

Results

Dose Response Relationship of male and female C57BL6 mice after exposure to PBI/BM5

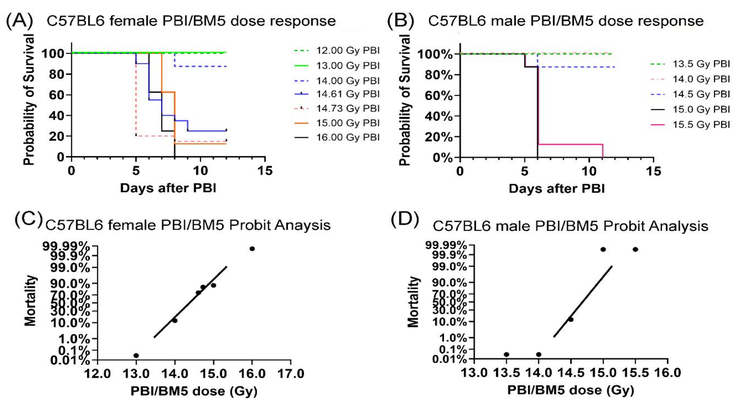

Fig. 2. Dose response relationship of female and male C57BL6 after PBI/BM5. (A) Kaplan-Meier survival curves of female C57BL6 after PBI/BM5. (B) Kaplan-Meier survival curves of male C57BL6 after PBI/BM5. (C) Probit plot showing the DRR of female mice exposed to PBI/BM5. (D) Probit plot showing the DRR of male mice exposed to PBI/BM5.

Results

Sources of cytokine variance in irradiated male and female mice

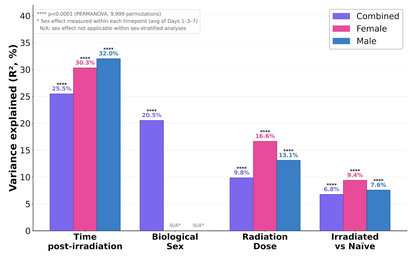

Fig 3. Biological sex explains ~20% of variance in radiation-induced cytokine profiles. PERMANOVA analysis of 65 serum cytokines comparing male and female mice at each study time point (Day 0–7 post-irradiation). Irradiated samples were normalized to sex-matched naïve baselines ($\log_2$FC vs. naïve) prior to analysis. The R^2 value represents the proportion of total cytokine variance attributable to biological sex. Analysis was performed using Euclidean distance with 9,999 permutations.. **** p<0.0001.

Sex differences in baseline cytokine profiles

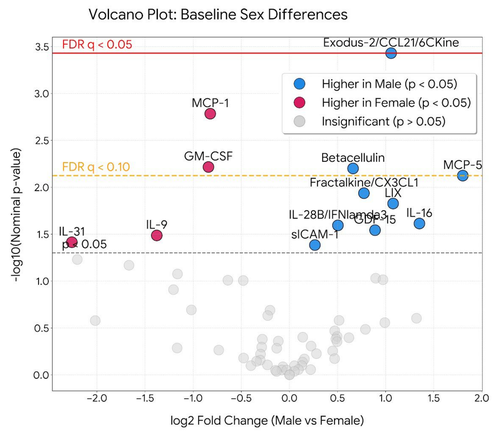

Fig. 4 Baseline sex differences in serum cytokine profiles of naïve male and female mice. Volcano plot showing cytokine differences between naïve female and male mice. The x-axis represents $\log_2$ fold change (female vs. male); the y-axis represents $-\log_{10}$(nominal p-value). Dashed orange line: nominal p=0.05; dashed purple line: FDR-adjusted p=0.05. Thirteen cytokines reached nominal significance (p<0.05); Exodus-2/CCL21 was the only cytokine to remain significant after Benjamini–Hochberg FDR correction (q<0.05). Independent samples t-test, n=4/sex.

Cytokine PCA by sex across post-irradiation time points

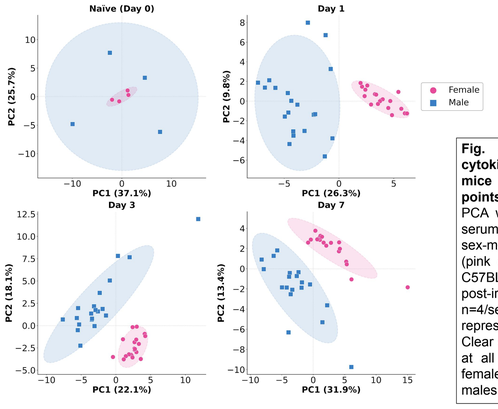

Fig. 5. PCA of radiation-induced cytokine profiles in male and female mice across post-irradiation time points. PCA was performed on 65 normalized serum cytokines ($\log_2$FC vs. sex-matched naïve baseline) in female (pink circles) and male (blue squares) C57BL/6 mice at Days 0, 1, 3, and 7 post-irradiation (n=20/sex/time point; n=4/sex for naïve). Shaded ellipses represent 95% confidence intervals. Clear sex-based separation is observed at all post-irradiation time points, with females forming tighter clusters than males..

Sex-specific and shared cytokine responses to high-dose partial-body irradiation

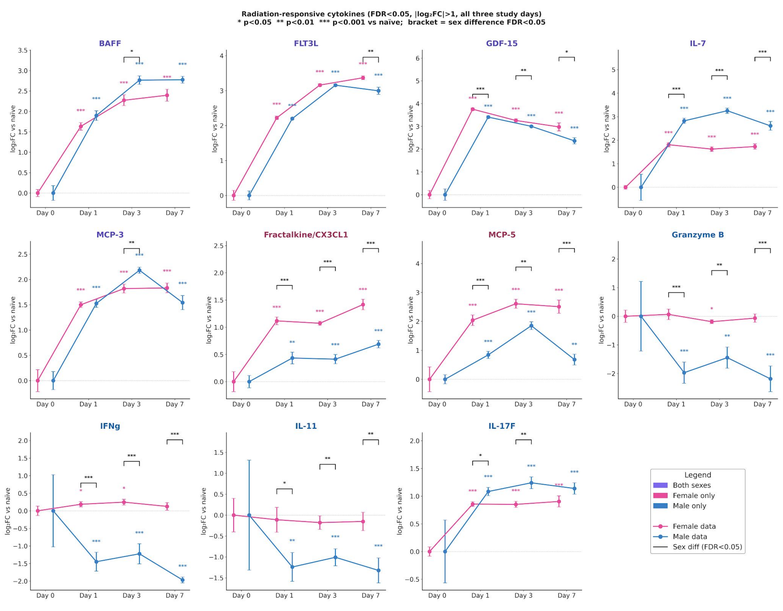

Fig 6. Divergent and conserved cytokine responses to radiation in male and female mice. Line graphs showing mean (±SEM) $\log_2$ fold change vs. sex-matched naïve baseline for each cytokine, at Days 0, 1, 3, and 7 post-irradiation. Pink circles: female mice; blue squares: male mice. Cytokines were included if they met FDR q<0.05 and $|\log_2 FC|>1$ (≥2-fold change) in both sexes at all three post-irradiation time points. Asterisks indicate significant change from naïve baseline within each sex (* FDR q<0.05, ** q<0.01, *** q<0.001). Brackets with asterisks indicate significant differences between sexes at the indicated time point (FDR q<0.05). n=20/sex/time point (4 mice × 5 doses).

Dose-responsive cytokines at Day 7: shared and sex-specific

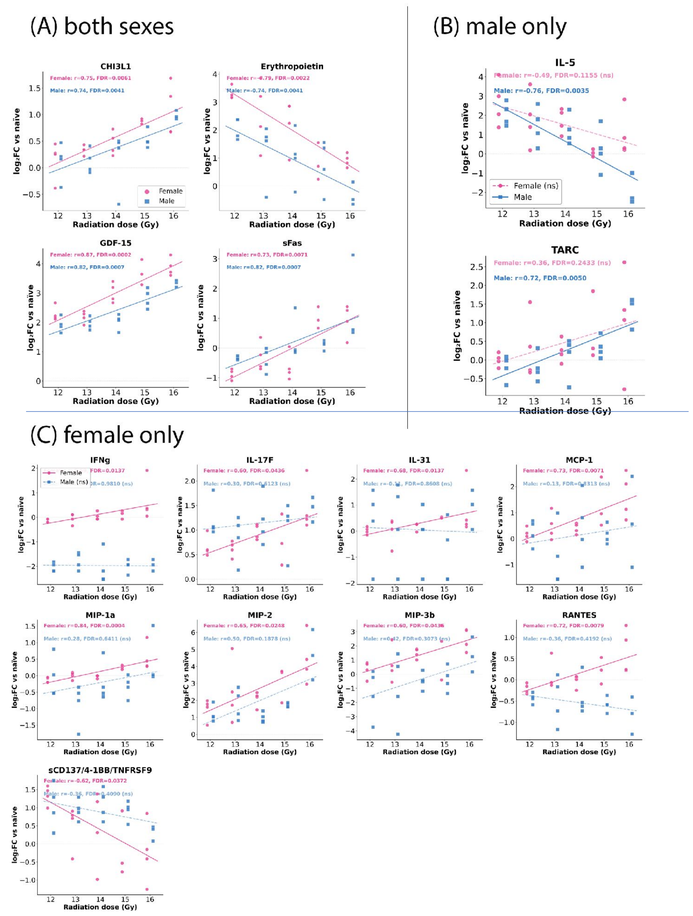

Fig. 7 Cytokines showing dose-dependent responses on day 7 post radiation in both sexes, or single sex only. (A) Cytokines that are dose-dependent responsive in both sexes. (B) cytokines that are dose-dependent responsive in male mice only. (C) cytokines that are dose-dependent responsive in female mice only. No cytokines showed dose response on d1 or d3. Line graphs showing mean (±SEM) $\log_2$ fold change vs. sex-matched naïve baseline for each cytokine, on day 7 post-irradiation. Pink circles: female mice; blue squares: male mice. Cytokines were included if they met FDR q<0.05 and $|\log_2 FC|>1$ (≥2-fold change) in both sexes at all three post-irradiation time points. Asterisks indicate significant change from naïve baseline within each sex (* FDR q<0.05, ** q<0.01, *** q<0.001). Brackets with asterisks indicate significant differences between sexes at the indicated time point (FDR q<0.05). n=20/sex/time point (4 mice × 5 doses).

Conclusions

- Biological sex is a major determinant of radiation-induced cytokine responses, independently explaining ~20% of total cytokine variance at all post-irradiation time points
- A conserved five-cytokine core signature (BAFF, FLT3L, GDF-15, IL-7, MCP-3) was consistently upregulated in both sexes across all three study days, representing robust sex-independent radiation biomarker candidates
- Males showed sustained suppression of IFNγ, Granzyme B, and IL-11 across all time points, indicating sex-specific attenuation of cytotoxic immune effector activity following high-dose irradiation
- Females demonstrated sustained and stronger upregulation of Fractalkine/CX3CL1 and MCP-5, suggesting enhanced chemokine-driven immune recruitment responses
- Radiation dose-dependent cytokine responses were minimal on Days 1 and 3, but emerged strongly at Day 7, with GDF-15 identified as the most robust dose-tracking biomarker in both sexes
- Female mice showed greater dose-discriminating capacity at Day 7 (13 dose-responsive cytokines) compared to males (6 cytokines), suggesting sex influences the performance of cytokine-based biodosimetry
- These findings demonstrate that sex must be incorporated as a biological variable in radiation biomarker discovery, biodosimetry model development, and medical countermeasure evaluation

Discussion

- Normalization to sex-matched naïve baselines was essential to isolate true radiation-induced cytokine changes from pre-existing sex differences, underscoring the importance of sex-matched controls in radiation biomarker studies
- The conserved five-cytokine signature (BAFF, FLT3L, GDF-15, IL-7, MCP-3) likely reflects shared hematopoietic injury and immune mobilization responses independent of sex-specific immune programming
- Sustained suppression of IFNγ and Granzyme B in males suggests radiation-induced attenuation of cytotoxic immune activity, which may contribute to sex differences in susceptibility to radiation-induced immune complications
- Enhanced upregulation of Fractalkine/CX3CL1 and MCP-5 in females is consistent with stronger innate immune activation and enhanced leukocyte trafficking responses reported in females
- The emergence of dose-dependent responses at Day 7, but not Days 1–3, suggests early cytokine release reflects the systemic response to radiation exposure, while later responses mirror cumulative tissue damage and hematopoietic suppression
- GDF-15 emerged as the strongest sex-independent dose-tracking biomarker (r=0.82–0.87, Day 7), supporting its potential utility in biodosimetry applications
- Small group sizes (n=4/dose/sex) and a single mouse strain limit generalizability; validation in larger, more diverse cohorts is warranted

References

1. N. Staff, P. Biodosimetry Blue Ribbon Panel, NIAID Biodosimetry Strategic Plan. *Radiation research* **205**, 199–205 (2026).
2. H. M. Swartz, A. B. Flood, Rethinking the Role of Biodosimetry to Assess Risks for Acute Radiation Syndrome in Very Large Radiation Events: Reconsidering Legacy Concepts. *Radiation research* **201**, 440–448 (2024).
3. W. Cui *et al.*, Cytokine Profiling for the Prediction of Lethality and High-Dose Exposure in a Murine Partial Body Irradiation Model. *International journal of molecular sciences* **27**, (2026).
4. Y. Wu *et al.*, Contribution of immune cells and cytokines to radiation-induced injury: the key roles and potential targets. *Frontiers in immunology* **17**, 1785418 (2026).
5. J. G. Kiang, G. Cannon, An Update on Dynamic Changes in Cytokine Expression and Dysbiosis Due to Radiation Combined Injury. *International journal of molecular sciences* **26**, (2025).
6. M. M. Satyamitra, D. R. Cassatt, L. Marzella, A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury. *Radiation research* **197**, 408–414 (2022).
7. D. R. Cassatt *et al.*, A Trans-Agency Workshop on the Pathophysiology of Radiation-Induced Lung Injury. *Radiation research* **197**, 415–433 (2022).
8. T. A. Winters *et al.*, Gastrointestinal Acute Radiation Syndrome: Mechanisms, Models, Markers, and Medical Countermeasures. *Radiation research* **201**, 628–646 (2024).
9. L. P. Taliaferro *et al.*, Sex differences in radiation research. *International journal of radiation biology* **100**, 466–485 (2024).
10. G. P. Holmes-Hampton, V. P. Kumar, K. Valenzia, S. P. Ghosh, Sex as a factor in murine radiation research: implications for countermeasure development. *Cytogenet Genome Res*, (2023).

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