HHS Public Access

Author manuscript

Radiat Res. Author manuscript; available in PMC 2022 April 05.

Published in final edited form as:

Radiat Res. 2022 February 01; 197(2): 199–204. doi:10.1667/RADE-21-00142.1.

Re-examination of the exacerbating effect of inflammasome components during radiation injury

W. June Brickey[1], **Michael A. Thompson**[2], **Zhecheng Sheng**[3], **Zhiguo Li**[3,4], **Kouros Owzar**[3,4], **Jenny P.Y. Ting**[1,2,5,6]

[1]Department of Microbiology-Immunology, University of North Carolina at Chapel Hill, North Carolina, 27599, USA.

[2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, North Carolina, 27599, USA.

[3]Duke Cancer Institute, Duke University School of Medicine, Durham, North Carolina, 27705, USA.

[4]Department of Biostatistics & Bioinformatics, Duke University School of Medicine, Durham, North Carolina, 27705, USA.

[5]Department of Genetics, University of North Carolina at Chapel Hill, North Carolina, 27599, USA.

Abstract

Radiation can be applied for therapeutic benefit against cancer or may result in devastating harm due to accidental or intentional release of nuclear energy. In all cases, radiation exposure causes molecular and cellular damage, resulting in the production of inflammatory factors and danger signals. Several classes of innate immune receptors sense the released damage associated molecules and activate cellular response pathways, including the induction of inflammasome signaling that impacts IL-1β/IL-18 maturation and cell death. A previous report indicated inflammasomes aggravate acute radiation syndrome (1). In contrast, here we use heterozygous and gene-deletion littermate controls and find that inflammasome components do not exacerbate gamma irradiation-induced injury. Some inflammasome genes even result in radio-protection that is significant in male mice. We discuss parameters that may influence the role of inflammasomes as radio-protective or radio-exacerbating factors in recovery from radiation including the use of littermate controls, the sex of the animals, differences in microbiota within the colonies and other experimental conditions.

INTRODUCTION

Radiation can be applied for therapeutic benefit to destroy cancer cells or may cause devastating harm due to accidental or intentional release of nuclear energy. In all cases, radiation exposure causes DNA and cellular damage mediated through the production of

[6]***Corresponding Author*:** Jenny P.Y. Ting, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, Phone 919-966-5538, Fax; 919-966-3212, jenny_ting@med.unc.edu.

damage associated molecular patterns (DAMPs) (2, 3). These molecular danger signals, including nucleic acid molecules, ATP, HMGB1, heat shock proteins, oxidized protein and lipid fragments and metabolic factors, alert local and systemic immune responses to address radiation-induced injury and promote repair processes.

Several classes of pattern recognition receptors (PRR) sense the released DAMPs and activate cellular response pathways. These innate immune receptors include toll-like receptors (TLR), Nod-like receptors (NLR), RIG-I-like receptors (RLR) and C-type lectin receptors (CLR). Triggering these signaling pathways impacts subsequent production of inflammatory factors and activation of cell survival processes. To employ these pathways to mitigate damage induced by radiation, research has focused on characterizing biomolecules that engage PRR to provide radio-protection (4, 5).

The aim of this report is to examine the role of NLR in responding to radiation damage by focusing on the impact of loss of inflammasome in mice treated with total body irradiation. The inflammasome is a protein complex comprised of a receptor/sensor, the ASC common adaptor molecule and the executioner caspases (1 and 11 in mice). This leads to caspase-1 activation, resulting in pro-IL-1β, pro-IL-18 and pro-Gasdermin D (GSDMD) cleavage to mature IL-1β, IL-18 and GSDMD, causing inflammation and pyroptotic cell death (6).

The previous report by Hu et al. (1) describes the role of AIM2, ASC and caspase-1/11 in exacerbating subtotal body irradiation-induced gastrointestinal syndrome and total body irradiation-induced hematopoietic failure resulting from X-ray irradiation. In their study, wildtype C57BL/6N or C57BL/6J sub-strains were purchased from the National Cancer Institute or Jackson Laboratories and cohoused with *Casp1/11*$^{-/-}$, *Casp1*$^{-/-}$, *Asc*$^{-/-}$ (backcrossed to C57BL/6N) or *Aim2*$^{-/-}$ (backcrossed to C57BL/6J), and in each case, mice lacking the inflammasome gene showed a significant survival advantage over wildtype controls.

In contrast, we examined the impact of inflammasome genes against acute radiation injury induced by total body irradiation (TBI) by comparing co-housed littermates, to minimize genomic background and microbiota differences. We also separately studied males and females. For our studies, mice were subjected to cesium-sourced TBI (7.5 to 8.2Gy) and monitored for 30 days. Overall, we found that rather than exacerbating injury, inflammasome components are needed for protecting against the development of lethal acute radiation syndrome (ARS) from exposure to high doses of gamma-radiation.

MATERIALS AND METHODS

Mice

Gene deletion mice were backcrossed to C57BL/6J for more than 12 generations, housed in AAALAC-accredited specific pathogen free facilities and treated under protocols approved by the Institutional Animal Care and Use Committee at University of North Carolina at Chapel Hill. *Asc*$^{-/-}$ mice were obtained from V. Dixit (Genentech, San Francisco, CA) (7). *Casp1/11*$^{-/-}$ and *Nlrp3*$^{-/-}$ were as described (8). *Aim2*$^{-/-}$ mice were generated at UNC as described (9). Male and female mice (8–24 weeks of age) were age- and sex-matched

between gene-deficient and heterozygous littermates, subjected to TBI and provided feed and water *ad libitum* for 30 days of monitoring. Endpoint criteria included lethality, 25% weight loss, severe dehydration, agonal respirations, lying in prone position, or combinations of milder symptoms such as lesions, diminished activity, hunched posture or ruffled fur.

Radiation

Conscious mice were placed in 12-compartment lucite pie containers. The pie cages were placed in chamber of 137-cesium Irradiator (Gammacell 40 Exactor, Best Theratronics, Ontario, Canada) for TBI with gamma-ray doses of 7.5, 8.0 or 8.2Gy at a ~0.94Gy/min dose rate.

Statistics

Survival distributions were estimated using the Kaplan Meier method with differences analyzed using the log-rank (Mantel-Cox) test based on the corresponding asymptotic *P* value, employing a two-sided unadjusted significance level of 0.05. The analyses were performed using GraphPad Prism v9, with results from two to three replicate studies combined by genotype.

RESULTS

We investigated the role of inflammasomes in exacerbating or protecting against radiation-induced injury that results in ARS by subjecting mice lacking expression of inflammasome components to total body irradiation (TBI). First, we analyzed $Aim2^{-/-}$ mice, a focus of the Hu et al. report (1). AIM2 is a receptor of double stranded DNA and associates with ASC and caspases to perform its inflammasome function (10–13). $Aim2^{+/-}$ male mice presented a modest but statistically enhanced survival over $Aim2^{-/-}$ littermates at both 8.0 and 8.2Gy TBI (Fig. 1A, B), while no survival difference was seen among female $Aim2^{-/-}$ and $Aim2^{+/-}$ littermates given 8.2Gy TBI (Fig. 1C). We also analyzed NLRP3, which is the most studied inflammasome, and is also important for DNA-activated inflammasomes in both human and mice (14, 15). Male $Nlrp3^{+/-}$ mice appeared to have a modest but statistically significant survival advantage over male $Nlrp3^{-/-}$ littermates at 8.0 and 8.2Gy TBI (Fig. 1D, E), while no statistical difference in survival was observed between $Nlrp3^{+/-}$ and $Nlrp3^{-/-}$ female littermates at 8.2Gy TBI (Fig. 1F).

Next, we investigated the impact of loss of the commonly shared activating partners of the inflammasome complex, namely caspase-1/11 and Asc. Examination of $Casp1/11^{-/-}$ male mice revealed significantly reduced survival compared to $Casp1/11^{+/-}$ male littermates, while females showed no difference at 8.2Gy TBI (Fig. 2A, B). Similarly, $Asc^{+/-}$ displayed a statistically significant survival advantage over $Asc^{-/-}$ male littermates when exposed to 7.5Gy TBI, while females showed no difference (Fig. 2C, D). Overall, using a stringent *P* value of 0.05/8 or 0.00625 when comparing all genotypes, we found that Nlrp3 (at 8.0Gy), caspase-1/11 and Asc in males were significantly protective against H-ARS.

DISCUSSION

In summary, these results show that the absence of inflammasome does not protect against ARS, but the presence of inflammasome is correlated with a survival advantage in male mice. Many links of inflammasome activity to the control of inflammation and metabolism as well as to pathogenesis related to pathogen infection or immune dysregulation (autoimmunity) have been reported (16–18). In this study of littermate controls, we find that the inflammasome protects against radiation injury in male mice. It is possible that inflammasome sensing of danger signals resulting from gamma-irradiation stimulates immunity and directs responses toward recovery from, rather than aggravation of injury. The observations suggest the important potential for engaging the inflammasome pathway to induce protective mechanisms and factors against the damaging effects of radiation.

In contrast to a previous report (1), our study results do not indicate an exacerbating role for *Casp1/11, Asc, Nlrp3* or *Aim2* upon exposure to lethal dosages of TBI using a cesium source. Instead, all exhibited a protective role in males, but not in females, with radio-protection varying with gene deletion target. *Casp1/11* and *Asc*, which are shared by all or most inflammasome pathways, exhibited significant radio-protective effects, while the effects of *Aim2* and *Nlrp3* were much less prominent. In a companion report, Daniel et al.[7] also found a non-exacerbating role for *Casp1/11* deficient mice using X-ray irradiation approaches in both TBI and partial body radiation models. However, Daniel et al.[7] did not see a significant protective role of these two caspases. Although the effect of sex on mitigating responses to radiation has been demonstrated previously (19, 20), further investigation is required to fully understand the underlying mechanisms that cause differential responses in males and females.

Several explanations can be considered for the differences between our results and the Hu et al. report (1). One possibility is that we used cesium-based gamma-radiation, while Hu et al. (1) used X-ray radiation with the caveat that gamma radiation is more homogeneous than X-ray radiation. However, the Daniel et al.[7] study also used X-ray radiation and did not observe radio-protection by caspases-1/11 in male mice. Genetic drift is a less likely possibility because Hu et al. (1) observed the same phenotype across multiple genetically distinct strains obtained from different sources.

Another likely source of variation may be attributed to microbiota. Hu et al. (1) used C57BL/6N and C57BL/6J controls obtained from commercial sources, whereas we and Daniel et al.[7] used littermate controls to minimize differences in background genetics and microbiota. In addition to harboring distinct microbiome profiles, commercially sourced vs. in-bred mice differ in metabolomic profiles. While Hu et al. (1) conditioned their mice with two-week co-housing prior to testing, this duration may be insufficient to equalize the microbiota composition. Littermates originating from a common source should possess similar microbiota and alleviate concerns about any maternal effects. However, upon

[7]Companion Short Communication Submission: Daniel AR, Luo L, Lee CL, Kirsch DG. Investigating the Role of Inflammasome Caspases 1 and 11 in the Acute Radiation Syndrome. Radiat Res. 2021 Dec 1;196(6):686–689. doi: 10.1667/RADE-21-00141.1. PMID: 34644390; PMCID: PMC8665044.

communication with the authors of the Hu et al. report (1), they shared preliminary studies using littermates, but with a limited number of animals, replicating their earlier results that showed an exacerbating role for inflammasomes in X-ray radiation. Thus, an expanded, well-powered littermate study would be useful to elucidate answers to address contributions of microbiome.

Finally, the pathogen containment or cleanliness status may differ between facilities and between institutions such that the endemic microbiota in each animal facility (21) may uniquely and differentially influence the biologic impact of the inflammasome in response to radiation (22). Cross testing of institutionally derived microbiota would provide an opportunity to determine differential contributions of microbiota to responding to radiation injury. Additional remaining factors related to age of subjects, body weight or fat content related to metabolic parameters, time of day of radiation exposure and radiation doses (i.e. LD50 dose appropriate to male or female mice) may contribute to variable responses to radiation and should be considered in future investigations. Careful consideration of variables in well-controlled studies using genetically defined intact organisms (both male and female) is critical for validating cellular studies and elucidating the molecules, pathways and kinetics of injury and recovery responses to radiation.

ACKNOWLEDGMENTS

This work was funded by National Institute of Health grants R35 CA232109, U19 AI067798 and AI029564. The following disclosures are provided. JPYT is a cofounder of and stockholder in IMMvention Therapeutix, which is developing inflammasome inhibitors. JPYT is a cofounder and stockholder of Goldcrest Bio, which is developing mitigators of radiation adverse effects. WJB is also a stockholder of Goldcrest Bio.

REFERENCES

1. Hu B, Jin C, Li HB, Tong J, Ouyang X, Cetinbas NM, et al. The DNA-sensing AIM2 inflammasome controls radiation-induced cell death and tissue injury. Science. 2016;354(6313):765–8. [PubMed: 27846608]
2. Georgakilas AG, Pavlopoulou A, Louka M, Nikitaki Z, Vorgias CE, Bagos PG, et al. Emerging molecular networks common in ionizing radiation, immune and inflammatory responses by employing bioinformatics approaches. Cancer Lett. 2015;368(2):164–72. [PubMed: 25841996]
3. McBride WH, Chiang CS, Olson JL, Wang CC, Hong JH, Pajonk F, et al. A sense of danger from radiation. Radiation research. 2004;162(1):1–19. [PubMed: 15222781]
4. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 2008;320(5873):226–30. [PubMed: 18403709]
5. Kurkjian CJ, Guo H, Montgomery ND, Cheng N, Yuan H, Merrill JR, et al. The Toll-Like Receptor 2/6 Agonist, FSL-1 Lipopeptide, Therapeutically Mitigates Acute Radiation Syndrome. Scientific Reports. 2017;7(1):17355. [PubMed: 29230065]
6. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526(7575):660–5. [PubMed: 26375003]
7. Mariathasan S, Newton K, Monack DM, Vucic D, French DM, Lee WP, et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. Nature. 2004;430(6996):213–8. [PubMed: 15190255]
8. Jha S, Srivastava SY, Brickey WJ, Iocca H, Toews A, Morrison JP, et al. The inflammasome sensor, NLRP3, regulates CNS inflammation and demyelination via caspase-1 and interleukin-18. J Neurosci. 2010;30(47):15811–20. [PubMed: 21106820]

9. Wilson JE, Petrucelli AS, Chen L, Koblansky AA, Truax AD, Oyama Y, et al. Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis via DNA-PK and Akt. Nature medicine. 2015;21(8):906–13.
10. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature. 2009;458(7237):509–13. [PubMed: 19158676]
11. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 2009;458(7237):514–8. [PubMed: 19158675]
12. Jin T, Perry A, Smith P, Jiang J, Xiao TS. Structure of the absent in melanoma 2 (AIM2) pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly. The Journal of biological chemistry. 2013;288(19):13225–35. [PubMed: 23530044]
13. Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, et al. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell. 2014;156(6):1193–206. [PubMed: 24630722]
14. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nature reviews Immunology. 2019;19(8):477–89.
15. Gaidt MM, Ebert TS, Chauhan D, Ramshorn K, Pinci F, Zuber S, et al. The DNA Inflammasome in Human Myeloid Cells Is Initiated by a STING-Cell Death Program Upstream of NLRP3. Cell. 2017;171(5):1110–24.e18. [PubMed: 29033128]
16. Guo H, Callaway JB, Ting JPY. Inflammasomes: Mechanism of Action, Role in Disease, and Therapeutics. Nature medicine. 2015;21(7):677–87.
17. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature. 2012;481(7381):278–86. [PubMed: 22258606]
18. Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. Annu Rev Cell Dev Biol. 2012;28:137–61. [PubMed: 22974247]
19. Daniel AR, Lee CL, Oh P, Luo L, Ma Y, Kirsch DG. Inhibiting Glycogen Synthase Kinase-3 Mitigates the Hematopoietic Acute Radiation Syndrome in a Sex- and Strain-dependent Manner in Mice. Health physics. 2020;119(3):315–21. [PubMed: 32175929]
20. Jones JW, Alloush J, Sellamuthu R, Chua HL, MacVittie TJ, Orschell CM, et al. Effect of Sex on Biomarker Response in a Mouse Model of the Hematopoietic Acute Radiation Syndrome. Health physics. 2019;116(4):484–502. [PubMed: 30681425]
21. Chen L, Wilson JE, Koenigsknecht MJ, Chou WC, Montgomery SA, Truax AD, et al. NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth. Nature immunology. 2017;18(5):541–51. [PubMed: 28288099]
22. Guo H, Chou WC, Lai Y, Liang K, Tam JW, Brickey WJ, et al. Multi-omics analyses of radiation survivors identify radioprotective microbes and metabolites. Science. 2020;370(6516).

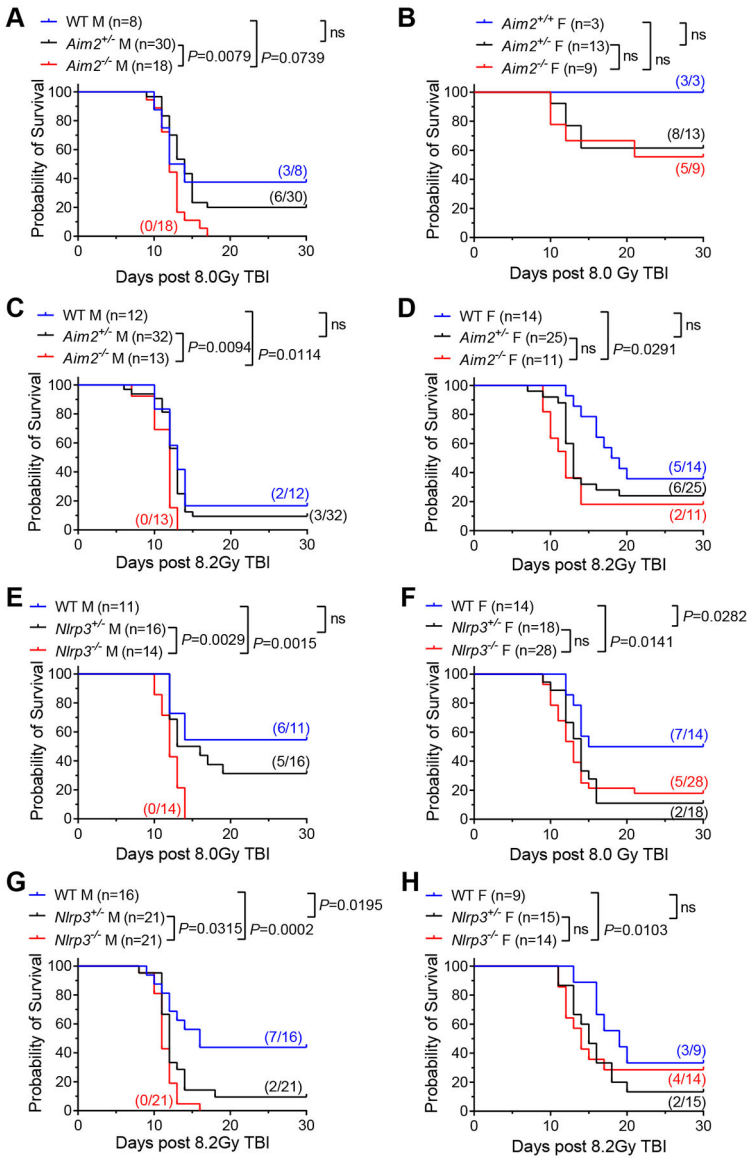

Fig. 1. Deletion of inflammasome components Aim2 and Nlrp3 do not protect against ARS.

The following animals were subjected to total-body irradiation (TBI): WT (not littermates of the next two groups), *Aim2*$^{+/-}$ and *Aim2*$^{-/-}$ littermate males **(panel A)** and females **(panel B)** at 8.0 or 8.2 Gy TBI **(panels C and D)**; WT (not littermates), *Nlrp3*$^{+/-}$ and *Nlrp3*$^{-/-}$ littermate males **(panel E)** and females **(panel F)** at 8.0 or 8.2 Gy TBI **(panels G and H)**. Mice were monitored for 30 days post irradiation. Survival distributions were estimated using the Kaplan Meier method where differences were examined using the log-rank (Mantel-Cox) test based on the corresponding asymptotic *P* value, employing a two-sided unadjusted significance level of 0.05. Multiple replicate studies with age- and sex-matched mice were combined with total animals (n) and 30-day survivors per total animals tested indicated within survival plots.

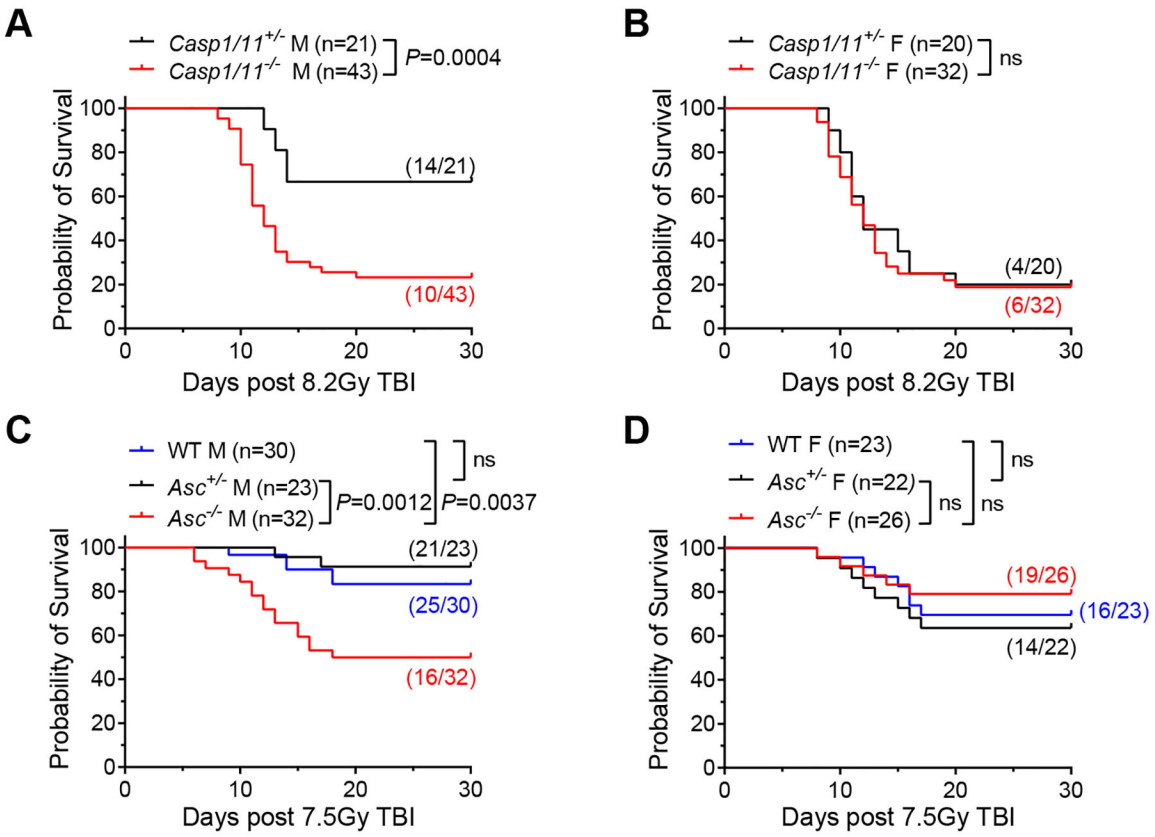

Fig. 2. Deletion of inflammasome components caspase-1/11 and Asc do not protect against ARS.

The following animals were subjected to TBI: *Casp1/11*$^{+/-}$ vs. *Casp1/11*$^{-/-}$ littermate males **(panel A)** or females **(panel B)** at 8.2 Gy TBI; WT (not littermates of the next two groups), *Asc*$^{+/-}$ vs. *Asc*$^{-/-}$ littermate males **(panel C)** or females **(panel D)** at 7.5 Gy TBI. Mice were monitored for 30 days post irradiation. Survival distributions were estimated using the Kaplan Meier method where differences were examined using the log-rank (Mantel-Cox) test based on the corresponding asymptotic *P* value, employing a two-sided unadjusted significance level of 0.05. Multiple replicate studies with age- and sex-matched mice were combined with total animals (n) and 30-day survivors per total animals tested indicated within survival plots.