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A Further Comparison of Pathologies after Thoracic Irradiation among Different Mouse Strains: Finding the Best Preclinical Model for Evaluating Therapies Directed Against Radiation-Induced Lung Damage

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Abstract

The human lung is among the most sensitive and critical tissues of concern in localized and systemic radiation exposures, and it is a subject of active preclinical research for evaluating mitigating therapies within the radiation countermeasures program. Our previous study comparing C57BL/6, CBA and C57L mice after whole-thorax irradiation pointed to the problems of late pleural effusions that prevented the full development of lung injury in C57BL/6 mice and suggested that the CBA and C57L strains are more favorable for modeling lung injury in humans (Jackson *et al.*, *Radiat. Res.* 173, 10–20, 2010). We extended these comparisons to include three other mouse strains (BALB/c, C57BR/J and A/J mice) irradiated with 10, 12.5 or 15 Gy. Most of these mice were unable to survive the first 6 months and presented with a mixture of lung injury and pleural effusions as determined from gross pathology, histology and micro-CT. The independent and varying development of compressive pleural effusions of ill-defined etiology represents a concern for these strains in that they may not satisfy the preclinical requirements for approval of medical countermeasures (e.g. radiation mitigators) for human use. Thus, among the various different mouse strains studied so far for these pathologies, only three (CBA, C3H and C57L) appear to be desirable in exhibiting an early wave of pulmonary dysfunction attributed exclusively to radiation pneumonitis and for further assessment of radioprotective and mitigating therapies. C57L mice are particularly relevant in that they show significant lung damage at lower radiation doses that are closer to what is predicted for humans.

INTRODUCTION

Radiation pneumonitis often presents as a major life-threatening complication within 6 months after either prescribed or accidental radiation exposures to relatively large volumes of the human lung (1–5). The pneumonitis (or alveolitis) reaction is characterized by a delayed but acute development of sterile inflammation involving initial damage to slowly proliferating resident epithelial and endothelial cells progressing to a complex interplay of inflammatory cell infiltrates and cytokines (6, 7).

A number of experimental studies on the rodent lung have been devoted to understanding the molecular and cellular mechanisms of radiation pneumonitis and fibrosis together with evaluation of agents that either have radioprotective properties when administered at the time of irradiation or serve as mitigators in targeting the postirradiation development of pulmonary pathology (8, 9). Many of the earlier investigations on mice in the 1980s used the C3H and CBA strains, and a discrete wave of lethal pneumonitis was observed after whole-lung irradiation, showing a latency and time course within 6 months that resembled the picture seen in humans (10–12). Diverse strain-related responses were also recognized in which the C57BL strains showed a less defined period of pneumonitis and a prolonged latency beyond 6 months (13, 14). The documentation of focal fibrosis in the lungs of C57BL/6 mice at the time of late mortality (15, 16) has subsequently encouraged a transition to the point that the majority of radiation lung experiments are now conducted on this strain, including those that fall under the radiation countermeasures program (9). Nonetheless, our recent study has questioned the reliability of C57BL/6 mice as a model of radiation-induced lung damage because overt pneumonitis is poorly defined in this strain, even at high radiation doses, and survival of many of the mice is limited by the development of large accumulations of pleural fluid that appear to be unrelated to either pneumonitis or fibrosis (17). The same study, however, showed that CBA and C57L mice exhibit a predictable and earlier pneumonitis, with a temporal onset similar to that observed in humans. The lungs of the C57L strain are especially interesting because they are the most sensitive after radiation exposure to either the whole thorax or the whole body, with the pneumonitis progressing to a pathology of fibrosis without the complications of pleural effusion (17, 18).

The purpose of the current study was to determine how three other mouse strains (BALB/c, C57BR and A/J mice) compare with respect to the timing and incidence of lung injury and pleural effusions after whole-thorax irradiation through evaluation of survival and measurements of lung and pleural fluid mass, micro-CT and histopathology. Each of these strains proved to be less desirable, because they presented with variable levels of pleural fluid accumulations concurrent with the early development of pneumonitis, leaving C3H, CBA and C57L mice as the least complicated for further studies on radiation lung injury.

MATERIALS AND METHODS

Male BALB/c (BALB/cAnNCrI, Charles River Laboratories International, Inc., Wilmington, MA), C57BR/cdJ and A/J mice (Jackson Laboratory, Bar Harbor, ME) were housed under identical conditions in approved facilities at the Massachusetts Institute of Technology (MIT). Facilities were free of known pathogenic organisms, and experiments were approved by the Institutional Animal Care and Use Committee at MIT. At 12 weeks of age, the thoraces of mice were singularly irradiated at doses of 10 Gy (BALB/c, $n = 4$), 12.5 Gy (BALB/c, $n = 5$ and C57BR, $n = 4$), and 15 Gy (A/J, $n = 4$) using ^{137}Cs γ rays (GammaCell 40, Atomic Energy Limited, Ottawa, Canada) at a dose rate of 0.51 Gy min^{-1} as described previously (17). The mice were irradiated without anesthesia and were contained within holders as described previously (19).

The physical condition of the mice was closely monitored and animals were euthanized when they entered respiratory distress as determined by labored breathing, lethargy and depressed rectal temperature ($<34^\circ\text{C}$). After the mice were euthanized, the thorax was carefully opened and the pleural fluid and lung tissue were weighed as described previously (17). The lungs were then inflated with 10% neutral buffered formalin, processed for histology and stained with Masson's trichrome.

X-ray micro-computerized tomography (micro-CT) was performed on mice at 18 weeks under isoflurane anesthesia using an eXplore Locus Micro CT Scanner with MicroView 2.1.

software (GE Medical Systems, London, ON, Canada) and the lung parenchyma density was determined in Hounsfield Units (HU) as described by Jackson *et al.* (17).

T2-weighted magnetic resonance imaging (MRI) (coronal and axial) was performed, also as described by Jackson *et al.* (17), using a 4.7-T MRI machine (Bruker BioSpin Corporation, Billerica, MA) with a mouse body coil.

RESULTS

All BALB/c, C57BR and A/J mice survived up to 18 weeks after exposure to 10–15 Gy ^{137}Cs γ rays followed by a rapid decline in survival, with only one of four mice BALB/c mice receiving 10 Gy remaining alive at the termination of the experiment at 28 weeks postirradiation (Fig. 1).

Increased lung weight, an indicator of edema and inflammation, was evaluated along with pleural fluid accumulation within the thoracic cavity. Figure 2 displays the lung and pleural fluid mass of individual BALB/c, C57BR and A/J mice as a function of the time at which they were euthanized due to respiratory distress. Also included for comparison are the data from Jackson *et al.* for C57L, CBA and C57BL/6 mice (17) that were treated and evaluated under the same conditions. Both parameters showed above normal values but with a wide scatter between 18 and 25 weeks for all BALB/c, C57BR and A/J mice. According to the two-tailed non-parametric Mann-Whitney *U* test, no significant difference ($P > 0.05$) in lung mass was observed between BALB/c mice irradiated at 10 and 12.5 Gy or between BALB/c and C57BR mice, while BALB/c mouse lung weights were significantly higher than those of A/J and C57BL/6 mice ($P < 0.01$). The macroscopic appearance of the lung tissue (reddening and consolidation) was also variable and followed the increase in lung mass. For pleural fluid weights, no differences ($P > 0.05$) were found between the two radiation doses delivered to BALB/c mice or between BALB/c, C57BR and A/J mice. However, the pleural fluid values for BALB/c mice were significantly higher than those for CBA ($P < 0.05$) or C57L ($P < 0.01$) mice and significantly less than those for C57BL/6 mice ($P < 0.01$).

Histological sections of lung tissues from eight BALB/c mice experiencing respiratory distress at 18 to 24 weeks after 10 or 12.5 Gy whole-thorax irradiation were reviewed, and all showed typical features of pneumonitis (alveolitis), with abundant infiltrates of mononuclear cells and alveolar macrophages, interstitial and intra-alveolar edema and lymphocytic perivascular cuffing (Fig. 3B), as described previously for this and other mouse strains (14). Focal areas of mild fibrosis were also noted in three BALB/c mice. Pneumonitis was similarly seen histologically in four A/J mice (Fig. 3C) at 20 to 25 weeks after 15 Gy and four C57BR mice (Fig. 3D) at 21 to 26 weeks after 12.5 Gy. The appearance of elongated crystalline material in the terminal bronchioles and alveolar ducts was an additional finding in the C57BR mice, as reported for C57L mice (17, 18).

Increased lung density on micro-CT images was observed among irradiated BALB/c, C57BR and A/J mice both visibly (Fig. 4A) and quantitatively (Fig. 4C) at 18 weeks, just prior to the onset of mortality. Also shown in Fig. 4C are the mean CT lung densities as a function of radiation dose for C57L, CBA and C57BL mice as reported by Jackson *et al.* (17). This shows that the dose-dependent sensitivity of BALB/c and C57BR mice to lung density changes are similar to that of C57L mice. However, the BALB/c and C57BR mouse strains differ from C57L mice in that pneumonitis is often accompanied by pleural effusions and that both can contribute to increased lung density. The combined effect of these pathologies is more clearly appreciated from T2-weighted MRI, where an enhanced signal is evident as fluid above the diaphragm as well as within the lung tissue (Fig. 4B).

DISCUSSION

Large accumulations of pleural fluid represent an anomaly in rodents after whole-thorax irradiation and interferes with the direct assessment of radiation injury to the lung (17). Pleural effusions are not a pathology typically reported in humans after thoracic irradiation. Pleural effusions on the scale observed in this study and our previous study (17) can prevent the full expansion of the lungs during respiration and may play a significant role in pulmonary dysfunction and animal survival after radiation exposure. In the present study, we sought to expand upon our previous study of three mouse strains (C57BL/6, CBA and C57L) by including three others (BALB/c, C57BR and A/J) in which such a problem might be avoided. Our investigation of BALB/c mice is of interest because this strain is well recognized to be radiosensitive for a number of different tissues and is genetically predisposed to enhanced radiation-induced genomic instability through lowered DNA-PK activity (20, 21). We did find that BALB/c mice exhibited a high sensitivity to whole-thorax irradiation given that a dose as low as 10 Gy proved to be lethal, which is in keeping with previous results for this strain (14, 22). However, this seemingly low radiation tolerance of the lung should be tempered by the finding that pleural effusions often also arose in this strain with respiratory distress and may therefore have contributed to mortality as an extraneous complicating factor. This phenomenon also appeared to apply to a variable extent to the other mouse strains used in the present study, C57BR and A/J. A survey of the literature shows that an early incidence of pleural effusions was also seen (before 6 months) in SWR, CF1, WHT and TO mice (14, 23, 24). An even earlier development of effusions after whole-thorax irradiation has been documented after higher doses in Brown Norway, WAG and August rats (24–26).

The coincident development of pneumonitis and pleural effusion in these rodent strains may give the impression that the complications are somehow causally related. Comparisons with CBA and C3H mice, however, show that lethal pleural effusions can arise after a much longer latent period (>6 months) when pneumonitis has completely subsided (12, 13, 27, 28). CBA and C3H mice also show minimal or absent fibrosis at this time (13, 15, 16, 27, 28). Thus the evolution of effusions appears to have little bearing on any injury inflicted on the lung parenchyma. Analysis of pleural fluid protein content in CBA mice points to an exudate rather than a transudate (28), making cardiac damage unlikely as a cause. While the etiology of effusions has yet to be defined, its overall presentation is consistent with separate damage to the draining lymphatics of the pleurae as very occasionally seen in patients receiving thoracic radiotherapy (29–31).

An overview of the different mouse and rat strains in terms of the timing of pneumonitis and pleural effusions after whole-thorax irradiation is shown in Fig. 5, which includes the earlier results of Jackson *et al.* (17). This overview shows how the two types of pathologies can vary independently with respect to their latency and encourages the concept that they evolve from radiation sterilization of separate target cell populations with perhaps genetically determined differences in cell turnover. The dissociation of pneumonitis and pleural effusions are further seen in their independent radiation dose modification in CBA mice by anesthesia or amifostine radioprotection and continuous low dose rate (19, 27, 28). Given that the latent period before the onset of pleural effusions is also inversely related to radiation dose (13), we can now envisage how the effusions would dominate and mask the amelioration of radiation lung damage in situations in which a therapy has been selectively effective at protecting or mitigating against pneumonitis. This potential problem and its impact on estimated dose-modifying factors with elevation in iso-effective radiation dose is displayed hypothetically in Fig. 6 for five categories of mouse strains as well as for rats. Thus the most reliable experimental data would come from irradiation of C57L mice because of their high sensitivity in exclusively developing pneumonitis with moderate

fibrosis. CBA or C3H mice may also provide meaningful data, although this may necessitate a re-evaluation of the time of assessment if the pleural effusions appear earlier when the radiation dose increases, as exemplified by radioprotection with anesthesia (19). C57BL/6 mice appear to have an extraordinarily long latency for developing pneumonitis that can be better defined when avoiding pleural effusions through hemithoracic irradiation (32). Examples in which resolution of radiation lung injury has been observed at the histological level while having little impact on survival of C57BL/6 mice after whole-thorax irradiation have come from studies on genetic deficiencies in ICAM-1 (33) or integrin $\alpha\text{v}\beta 6$ (34) and mitigation with antioxidant enzymes (35). The hybrid cross of CBA/Ca and C57BL/Cbi (CBBF1) imparts a latent period for pneumonitis that is intermediate between those of the two parent strains (24, 32). While the time course of mortality or lung dysfunction may appear similar to those of C57L, CBA and C3H mice, the degree of dose sparing with a therapeutic agent may still be underestimated in BALB/c, C57BR, A/J, WHT or TO mice because more mice succumb to the overlapping effusions than to pneumonitis. The picture in rats is different in that massive pleural effusions appear to arise only at higher doses (above 14 Gy) in at least three different strains (24–26) and precedes the full development of pneumonitis at about 2 months after whole-thorax irradiation. In this case, raising the radiation dose in an effort to achieve equivalent level of lung damage will be frustrated by early mortality.

Also included in Fig. 6 for all categories is a very early subclinical response, because histological changes have been observed within days after radiation exposure to the lungs in at least C57BL/6 mice (36–38), and they appear to be accompanied by local production of certain cytokines (39, 40). Many of the existing mechanistic concepts start with these very early radiation-induced changes in the lung, but they are highly speculative because it is still uncertain as to how such a transient reaction heralds the long-term development of pneumonitis or fibrosis. The diverse manifestation of lung injury among the different rodent strains may therefore help to clarify the significance of the sub-acute pulmonary responses but with the caveat that pleural effusions not interfere with extrapolation to late effects.

To summarize our investigations of mice after whole-thorax irradiation, which now covers seven different genetic strains with monitoring of the impact of pleural fluid accumulation as well as pneumonitis, we found that only three of these strains (C57L, CBA and C3H) meet the requirement of producing clinically relevant pathology that satisfies their use in testing therapies aimed at preventing pulmonary complications in wide-field radiotherapy and nuclear incidents.

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FIG. 1.

Percentage survival as a function of time after whole-thorax irradiation with ^{137}Cs γ rays to BALB/c mice at 10 Gy ($\blacktriangledown$) and 12.5 Gy ($\bullet$), C57BR mice at 12.5 Gy ($\blacksquare$), and A/J mice at 15 Gy ($\blacktriangle$).

FIG. 2.

Fresh lung mass (panel A) and pleural fluid content (panel B) from individual BALB/c (●), C57BR (■) and A/J (▲) mice. All animals showed external damage to the lungs (focal to diffuse reddening and consolidation) but many also presented with pleural effusions. Shown for comparison are data from C57L (△), CBA (○) and C57BL/6 (□) mice exposed to ^{137}Cs γ rays from Jackson *et al.* (17). Shaded area represents the standard deviation (SD) around the mean of five unirradiated control mice. WTI, whole-thorax irradiation.

FIG. 3.

Masson's Trichrome-stained lung sections from different mouse strains. Panel A: Healthy unirradiated control BALB/c mouse with normal tissue architecture. Lung weight = 195 mg, pleural fluid = 0.02 g. Panel B: BALB/c mouse irradiated with 10 Gy with respiratory distress at 22 weeks showing inflammatory pneumonitis (alveolitis). Lung weight = 471 mg, pleural fluid = 1.13 g. Panel C: A/J mouse irradiated with 15 Gy with respiratory distress at 25 weeks. Lung weight = 311 mg, pleural fluid = 0.81 g. Panel D: 1 C57BR mouse irradiated with 12.5 Gy with respiratory distress at 22 weeks with crystalline material (CM) in bronchiole as well as pneumonitis. Lung weight = 448 mg, pleural fluid = 0.65 g. Bars represent 200 μm.

FIG. 4.

Panel A: Micro-CT coronal and axial sections through the mid-thorax of control and irradiated BALB/c mice at 18 weeks after 12.5 Gy whole-thorax irradiation to show regional increase in lung parenchyma density (arrows). Panel B: Coronal and axial T2-weighted MRI of the same irradiated BALB/c mouse showing increase signal within the lung indicative of edema and a pleural effusion above the diaphragm. Panel C: Quantitative CT analysis of lung density at 18 weeks as a function of radiation dose for BALB/c (●), C57BR (■) and A/J (▲) mice. Also shown for comparison are data from C57L (△), CBA (○) and C57BL/6 (□) mice from Jackson *et al.* (17). Error bars represent ± 1 SEM for 3 to 5 mice per irradiation group.

FIG. 5.

The varying development of radiation pneumonitis (Pn) and pleural effusions (PE) among different rodent strains after thoracic irradiation (17) with inclusion of the BALB/c, C57BR and A/J mice in the present study.

FIG. 6.

Selective protection/mitigation of pneumonitis after whole-thorax irradiation can be masked by pleural effusions depending on rodent strain. Hypothetical development of the pneumonitis and pleural effusion phases is shown for five categories of mice and for rats where the intrusion of effusions can affect the radiation dose–incidence curve for, e.g., survival and lung function and cause an underestimation of dose sparing by a protector or mitigator capable of reducing pneumonitis. A very early and subclinical response is also indicated as seen in temporal histological changes and expression of cytokine genes, but it remains unknown whether this has any predictive or causal relationship with salient injury among the different strains.