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Early Alterations in Cytokine Expression in Adult Compared to Developing Lung in Mice after Radiation Exposure

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Abstract

To assess early changes in the lung after low-dose radiation exposure that may serve as targets for mitigation of lung injury in the aftermath of a terrorist event, we analyzed cytokine expression after irradiation. Adult mice were studied after whole-lung or total-body irradiation. Mouse pups of different ages were also investigated after total-body irradiation. mRNA abundance was analyzed in tissue and plasma, and pathological changes were assessed. In lung tissue, dose-related changes were seen in IL1B, IL1R2 and CXCR2 mRNA expression at 1 and 6 h after irradiation, concurrent with increases in plasma protein levels of KC/CXCL1 and IL6. However, in the pups, changes in IL1 abundance were not detected until 28 days of age, coincident with the end of postnatal lung growth, although apoptosis was detected at all ages. In conclusion, although cytokines were expressed after low doses of radiation, their role in the progression of tissue response is yet to be determined. They may be candidates for use in marker-based biodosimetry. However, the lack of cytokine induction in early life suggests that different end points (and mitigating treatments) may be required for children.

Introduction

Since September 11, 2001, there has been increased awareness of the risk of a domestic radiological or nuclear event, potentially involving mass casualties (1–3). After such an occurrence, mobilizing a rapid medical response for those affected by acute radiation effects will obviously be of immediate and paramount importance. Fortunately, outcomes from some recent accidental events, notably Chernobyl and Tokai-mura (4,5), suggest that modern medical efforts are able to support the majority of victims through the acute radiation syndrome (ARS) if sufficient supplies and emergency medical personnel are available. However, these measures have proven ineffective in preventing the subsequent progression of radiation-induced lung effects, notably radiation pneumonitis (6,7), highlighting the need to develop specific agents targeted at such late consequences,

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including in that segment of the exposed population that receives non-lethal doses, which may occur in members of the exposed population who receive doses that may not be lethal soon after irradiation. Given that earlier studies suggested that such mitigating efforts require administration to be initiated as quickly as possible after irradiation (8–10), it seems likely that acute changes in pulmonary cytokine expression that may be critical to or affect the development of late effects could be targets for such mitigation. Serendipitously, these cytokine changes also may be of use to those working on the identification of biomarkers that could be used to identify the population at risk.

Classical studies of radiation-induced pulmonary late effects have suggested a temporal sequence of events that consists of an early “latent” period, a later persistent chronic inflammation, followed by collagen deposition and increasing extracellular matrix deposition, culminating in fibrosis (11–14). Such studies have supported the hypothesis that chronic inflammation resulting from the sequential and cyclical release of cytokines, growth factors and chemokines stimulates development of the fibrosis and that this mediator expression begins within the period immediately after radiation injury (15–17). Our group and others have shown that the underlying temporal sequence that culminates in late radiation effects in the lung involves both an acute (and persistent) expression of such proinflammatory cytokines as tumor necrosis factor A (TNFA) and interleukins IL1A and IL1B (18,19). Therefore, as part of our investigation into the pulmonary consequences of a radiological terrorism event, we examined early cytokine expression after single low doses of radiation in our mouse model, looking at those mediators that can be detected in the peripheral circulation as well as those expressed directly in the lung tissue. The aim of this initial investigation was to establish cytokine expression as an indicator of pulmonary radiation exposure, with the anticipation that such expression may provide targets for mitigation.

A mass casualty event will likely affect a broad spectrum of the population; therefore, markers of radiation exposure will need to be applied to populations other than the standard “healthy adult”. The response of children to radiation-induced damage is a relatively unexplored area, despite this population clearly being at greater risk due to their increased sensitivity (20–22). A significant portion of lung development takes place postnatally. During this period, which in humans involves the first 6 to 8 years of childhood (23), the lung is undergoing alveolarization and continued morphogenesis, including differentiation of critical cell types and systems, among which are the respiratory epithelium and critical immune effector cell populations (24). Factors that disrupt these developmental events have been shown to affect both the immediate and downstream responses of the pediatric lung (25); thus we hypothesized that children may demonstrate a differential cytokine expression, particularly with respect to the pro-inflammatory cytokines, after radiation exposure, resulting in altered outcomes, both acute and late. Therefore, we compared the cytokine expression after pulmonary irradiation in young mice, 4–28 days of age, as a model for the pediatric population.

Materials and Methods

Animals

Adult C57BL/6J mice (7–8-week-old females) were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were allowed to acclimate from shipping for 1 week prior to experimentation. Mice were maintained five per cage under filter caps in pathogen-free rooms and were supplied with standard laboratory diet and water *ad libitum*. C57BL/6 mice of both sexes [4, 7, 10, 14 and 28 (females only) days of age] were obtained from an in-house breeding colony. Pups were kept with their dams until weaning at 21 days of age; then

they were grouped by sex, five per cage. All treatment protocols were approved by the University of Rochester institutional animal care and use committee.

Radiation

Adults—Groups of 10 8-week-old C57BL/6J mice received either whole-lung or total-body irradiation (TBI) at doses ranging from 0–10 Gy from a ^{137}Cs γ -ray source operating at a dose rate of approximately 2.5 Gy/min. Mice were confined to plastic jigs and were not anesthetized during the procedure; age-matched control animals were sham-irradiated and maintained under identical conditions for the course of the experiment. Mice were killed between 1 h and 2 days after irradiation.

Neonates—Groups of five young C57BL/6 mice were placed in the same plastic jigs as the adults, either in their litter groups (before weaning) or in groups of five (after weaning), received 5 Gy TBI, and were killed 1 or 6 h after irradiation.

Peripheral Blood Analysis

Peripheral blood was collected by intracardial puncture from a minimum of five animals from each group and pooled for analysis. Samples were centrifuged at 6000g for 10 min, filtered through a 1.2- μm PVDF filter plate (Millipore), and then incubated overnight using a Beadlyte[®] Mouse Multi-Cytokine Beadmaster[™] Kit (Millipore) and accompanying Beadmates (Millipore). The cytokines analyzed included IL1A, IL1B, IL2, IL3, IL4, IL5, IL6, IL9, IL10, IL12 (p40), IL12 (p70), IL13, IL17, GM-CSF, IFN γ , KC/CXCL1, MCP1, MIP1B, RANTES, TNFA and VEGF. Cytokines were quantified using xMAP technology on a BioPlex 200 System (Bio-Rad Laboratories, WA).

Tissue Analysis

Histological preparation—After killing, the animals' lungs were inflated using 10% neutral-buffered formalin in a gravity perfusion apparatus. Additional target organs (spleen, gut and thymus) were dissected from the mice and all tissues were post-fixed for 16–24 h in 10% neutral buffered formalin. Tissues were dehydrated through graded alcohols, cleared through several changes of xylene, and infiltrated with paraffin. All tissues were embedded in paraffin, sectioned at 5 μm , and mounted on microscope slides.

Immunohistochemical techniques—Paraffin sections were deparaffinized in xylene and rehydrated through graded ethanol up to distilled water. Tissues for immunohistochemistry were pretreated in a citrate buffer (pH 6.0) for 38 min at 95–97°C in a water bath. After pretreatment, sections were incubated for 5 min with 3% H_2O_2 to inactivate endogenous peroxidases, then rinsed three times in Tris-buffered saline-Tween (TBS-T) containing 0.02% Triton X-100. Tissues then were blocked for 30 min at room temperature with a casein block followed by application of the primary antibody, which was applied to the sections and incubated at 4°C for 18 h; neutrophils were stained using rat anti-mouse neutrophil (Serotec). Slides were then rinsed three times in TBS-T and incubated with a biotinylated secondary antibody for 1 h at room temperature followed by another rinse and addition of a streptavidin-HRP tertiary reagent for 15 min at room temperature. Sections were reacted with substrate and chromagen (DAB) and counterstained with Lillie's modified hematoxylin and covered with cover slips.

TUNEL—DNA fragmentation was examined histologically using the terminal deoxynucleotidyl transferase (TdT)-mediated d-UTP nick end-labeling (TUNEL) technique. The ApopTag Plus Peroxidase In Situ Kit (Chemicon) was used for all TUNEL labeling. Tissues were digested using proteinase K for 1 min and then rinsed three times with TBS

buffer. Sections were then coated with TdT enzyme and incubated for 1 h at 37°C. After several washes in a stop buffer, a peroxidase-conjugated anti-digoxigenin antibody was applied to all sections and incubated for 30 min at room temperature. After subsequent washes, a peroxidase substrate was applied for 3–4 min then rinsed off with distilled water. Lung sections were counterstained with hematoxylin.

Tissue sections were imaged (Spot RT color, Diagnostic Instruments, Inc.) into 3 × 6 montages that were taken at 10× magnification to provide the greatest coverage of the entire lung sections. Six lung montages were analyzed and were counted manually for each dose and time. Counts of the TUNEL-positive cells were made at a magnification of 400%. Cells were scored as apoptotic only if they displayed condensed and fragmented chromatin.

Ribonuclease Protection Assay

Total RNA was isolated from lung tissue using TRIzol Reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Each frozen lung lobe (50–100 mg) was homogenized in 1 ml of TRIzol Reagent. Each final RNA pellet was resuspended in 50 µl of diethylpyrocarbonate-treated water. The RNA concentration and purity was quantified using the Gene Quant RNA/DNA Calculator (Pharmacia Biotech, Piscataway, NJ). Steady-state cytokine mRNA levels were quantified using a multi-cytokine ribonuclease protection assay (RPA), with custom riboprobe templates for IL1RI, IL1R2, IL10, IL1A, IL1B, IL1Ra, IL18/IGIF, IL6, L32 and GAPDH or TNFA, CCR1, CXCR2, TNFRp75, TNFRp55, CCR5, CCR2, IP-10, MCP1, CXCR3, L32 and GAPDH. The protected radiolabeled RNA fragments were electrophoresed on a 5% acrylamide/8 M urea sequencing gel, and the dried gel was used to expose X-AR film (Eastman Kodak, Rochester, NY) at –80°C with intensifying screens (Quanta III; Dupont, Wilmington, DE). For quantification, the dried gels were placed against PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA). The intensity of each specific chemokine and cytokine band was measured using a computer-linked PhosphorImager with ImageQuant software (Molecular Dynamics), and each intensity score was normalized to the intensity of hybridization for the constitutively expressed housekeeping genes GAPDH and L32 to correct for differences in loading. Quantification was performed using PhosphorImager counts rather than scanning densitometry since autoradiographic film has only an eightfold linear range whereas the PhosphorImager has a 100-fold range, allowing intensity measurements to be taken in the linear range for all messages measured.

Statistics

The relationships between cytokine expression levels and dose were examined using linear regression after logarithmic transformation, as described below. Expression levels across groups were compared using *t* tests. Results of RNase protection for the analysis of mRNA abundance were quantified by PhosphorImager analysis. Values for each mRNA species were normalized to the internal standards GAPDH or L32. Results of replicate experiments from five separate animals, analyzed in triplicate, were evaluated by ANOVA for independent measures, followed by Tukey or Dunnett tests of significance in multiple group comparisons. The two-tailed level of significance for all analyses was set at $P < 0.05$.

Results

Detection of Apoptosis Postirradiation

To assess the basic cell response to whole-lung and total-body irradiation, we performed TUNEL analysis on the lung tissue as a marker of apoptosis. As can be seen in Fig. 1, a clear apoptotic response was observed. Apoptosis peaked at 6 h postirradiation, although a

slightly earlier response was seen in the TBI mice compared to those receiving whole-lung irradiation alone (compare Fig. 2a and b).

Analysis of Cytokine Expression in Peripheral Blood in Adult Mice

We assessed cytokine expression in peripheral blood at times ranging from 1 h to 2 days after whole-lung and total-body irradiation using multiplex analysis. Of the 21 cytokines examined, only two showed evident dose- and time-related changes in expression, IL6 and KC/CXCL1; these changes were seen after both whole-lung irradiation (Fig. 3a and c) and TBI (Fig. 3b and d), with a dose-responsive increase in expression at 1 h that peaked at 6 h after irradiation and returned to baseline at 24 h; a more robust response was observed in the TBI animals.

To assess the significance of the changes in cytokine expression, plasma levels of IL6 and KC/CXCL1 were $\log_e$ -transformed so that the variances were approximately constant across dose. Since $\log_e(0)$ is undefined, 1 was added to each marker level and dose prior to taking the natural logarithm. Unexposed animals killed within 1 week after sham irradiation were included as controls for subsequent cytokine analyses. At 6 h, $\log_e(\text{IL6})$ levels were approximately the same for all doses ≤ 2 Gy (data not shown). $\log_e(\text{IL6})$ levels above 2 Gy were substantially and significantly higher than in the lower-dose group, although this higher-dose group showed little further increase with additional dose (Fig. 4a). Separate t tests confirmed that $\log_e(\text{IL6})$ levels among the animals exposed to 0.5 and 1 Gy were not significantly different from those for unexposed animals, whereas the $\log_e(\text{IL6})$ levels among animals exposed to 2.5, 5 and 10 Gy were each significantly different from those for unexposed animals. KC/CXCL1 levels increased with dose at both 1 and 6 h. At 1 h, $\log_e(\text{KC})$ increased approximately linearly with dose; the estimated slope (SE) was 0.20 (0.03) (Fig. 4b). At 6 h, $\log_e(\text{KC})$ was more linearly related to $\log_e(\text{dose})$ than to untransformed dose, although some departure from linearity was noted (Fig. 4c). The regression of $\log_e(\text{KC})$ on $\log(\text{dose})$ was significant, and the estimated slope (SE) was 1.46 (0.13). A two-sample t test comparing 6 h $\log_e(\text{KC})$ levels at low (≤ 2 Gy) and high (> 2 Gy) doses was also highly significant ($P < 0.0001$).

We also compared the 6-h expression levels of IL6 and KC/CXCL1 at low and high doses for animals exposed to total-body radiation. Figure 5 shows the comparisons for animals exposed to lung-only radiation (Fig. 5a and d) and total-body radiation (Fig. 5b and c). To facilitate comparison, Fig. 5a and d are the same as Fig. 4a. At 6 h after irradiation, animals exposed to high (> 2 Gy) and low (≤ 2 Gy) doses of total-body radiation had significantly different $\log_e(\text{IL6})$ expression levels (Fig. 5b). $\log_e(\text{KC})$ expression levels in low- (≤ 2 Gy) and high- (> 2 Gy) dose groups were significantly different both for animals exposed to lung-alone radiation (Fig. 5d) and for animals exposed to total-body radiation (Fig. 5c).

Analysis of Tissue mRNA Postirradiation

Using RPA technology, we identified a number of changes in mRNA expression in the lung tissue, notably in interleukin expression. After lung irradiation, significant increases were detected in the expression of IL1B at all doses 1 h after irradiation, with message induction ranging from three- to fourfold over the levels in sham-exposed mice (Fig. 6a and b). Similar responses were measured in total-body-irradiated mice at the same times, with induction of message ranging from three- to ninefold (Fig. 7a and b). At 6 h after irradiation, only the 5- and 10-Gy whole-lung-exposed groups demonstrated a significant message induction compared to sham-exposed mice (Fig. 6b), and levels in both groups had returned to baseline by 24 h after irradiation. No significant changes in IL1A message levels were measured after either exposure at any of the times or doses examined (Figs. 6b and 7b).

Significant increases in the expression of IL1R2 were also seen after whole-lung irradiation, for all doses, with message induction ranging from 3.7- to 4.7-fold over that for sham-exposed mice (Fig. 6a and c). More robust responses were measured in total-body-irradiated mice (four- to sixfold increases), with the exception of the lowest dose group, which was unchanged compared with sham-exposed mice (Fig. 7c). At 6 h after irradiation, only the 10-Gy whole-lung-exposed group demonstrated a significant (threefold) induction compared to sham-exposed mice (Fig. 6c). At 24 h after irradiation, both the whole-lung- and total-body-irradiated groups had returned to sham control levels at all doses. No significant changes in IL1RI message levels were measured at any of the times or doses examined.

Significant increases in the expression of the CCR1 and CXCR2 receptors were detected at 1 h after whole-lung irradiation at all doses examined, with message induction ranging from two- to fourfold and 2.5- to 5-fold, respectively, over that in sham-exposed mice (Fig. 8). By 6 h after irradiation, only the higher-dose (>2.5 Gy) groups demonstrated a significant message induction in CXCR2 compared to sham-exposed mice, and by 24 h after irradiation, message levels in all groups were similar to those of sham controls.

Image Analysis of Neutrophil Infiltration Postirradiation

Since both KC/CXCL1, a neutrophil chemotactic factor, and its receptor, CXCR2, had shown increased expression, the presence of neutrophils in the tissue was assessed using image analysis. Immunohistochemical staining confirmed the presence of neutrophils in the pulmonary parenchyma, with a significant increase in neutrophils at 6 h (Fig. 9), with the greatest response seen after 5 Gy. Immunohistochemical staining was also performed to assess changes in both macrophages and lymphocytes, but no changes in either population were observed.

Assessment of Age-Related Response: Adult and Mouse Pups

To assess whether age is a factor in early responses to radiation, mouse pups between 4 and 14 days old received total-body irradiation of 0 or 5 Gy and were killed at 1 or 6 h after irradiation. Since there was no statistically significant difference between the results for the various age groups ≤ 14 days of age, data for all pup groups was pooled and compared to data from 56-day (8-week)-old TBI animals.

Image analysis of TUNEL staining in the lungs indicated that, at both 1 and 6 h postirradiation, there was no significant difference in radiation-induced apoptosis between pups and adults (Fig. 10). Of note, the sham-irradiated pups had significantly higher apoptotic cell counts than the adults at both 1 and 6 h postirradiation. This result is consistent with observations by other investigators who have previously demonstrated two postnatal waves of programmed cell death in rodents, one beginning immediately after birth and another beginning in the third week (26,27), although there may have been additional stress due to handling and the transient removal from the dams. Only a limited analysis of changes in plasma cytokine abundance was possible due to the limited samples that could be obtained from the pups, and samples were pooled for animals 4–14 days of age. There appeared to be little difference with respect to age in the early KC/CXCL1 induction at 6 h postirradiation (Fig. 11). Unsurprisingly, given the postnatal waves of programmed cell death, when the neutrophil numbers were analyzed, the pups had significantly higher basal levels of neutrophils than the adult animals at both 1 and 6 h. In addition, the radiation-induced peak in neutrophil infiltration was seen earlier, at 1 h postirradiation (Fig. 12), although the numbers observed were statistically equivalent to the peak (6 h) response in the 56-day-old animals. Finally, the increase in abundance of mRNA for IL1B and IL1R2 seen in the 56-day-old animals at 1 h after irradiation was not detected in the 4–14-day-old pup groups (Fig. 13a and b). In an effort to identify the transition point for the interleukin

response, an older group of mouse weanlings (28 days of age) was studied. These animals demonstrated the anticipated increases in IL1B and ILR2 mRNA abundance of 2.8- and 2.2-fold, respectively, although that of IL1B was less robust. All other messages examined were unaltered with respect to sham control levels.

Discussion

Since September 11, 2001, both private and government-sponsored publications have suggested that, after either a large-scale radiological or nuclear event, there could be a rapid exhaustion of local resources and that successful management of any medical response will therefore require the ability to acquire biodosimetry for individuals and appropriately direct treatment (28). Treatment of those who have received doses that will induce acute lethal or severe responses will be of paramount importance. However, subsequent late effects may be seen not only among the survivors of acute injury but also in the vast majority of the population that received lower, sublethal doses. These may also require attention and possibly the use of mitigating therapies. Of the tissues/organs that could exhibit such late effects and therefore require preventative strategies, the lung has been identified as a prime candidate since both lethal and nonlethal radiation-induced pulmonary injuries have been seen after accidental exposures (7) and in the Japanese A-bomb survivors (29).

Similar to work from other investigators looking at normal tissue radiobiology, previous studies from our group using higher doses relevant to radiotherapy have shown that the lung responds rapidly, within hours, to radiation injury with increased expression of proinflammatory cytokines (18,19). This observation has led us to hypothesize that the immediate alteration in cytokine expression initiates and orchestrates a cascade of events involving an interaction between the injured tissues and infiltrating inflammatory cells (19,30), which culminates in the pulmonary late pathology. If this is true, such a sequence of events could be mitigated through interference in the cytokine-inflammation chain of communication, a supposition that was indirectly supported when we previously demonstrated the successful reduction of lung late effects through administration of lovastatin, a potent chemokine inhibitor (9).

However, application of such a hypothesis to the search for a mitigating regimen in the aftermath of a terrorist event holds a great deal of uncertainty since it is unclear whether the pulmonary response to low-dose radiation would result in a similar and immediate up-regulation of cytokine expression, with its subsequent inflammatory consequences, and a further complicating unknown, how total-body compared to whole-lung irradiation would affect such a response. Therefore, one of the aims of our project within the University of Rochester-based Center for Medical Countermeasures against Radiation (CMCR) was to assess pulmonary injury after either a localized or a systemic injury in terms of the tissue cytokine and inflammatory cell response. In addition, since other groups within the CMCR network are developing biodosimetric techniques for use as triage (31), we also looked at circulating proinflammatory cytokine levels with respect to their potential use as indicators and/or measures of radiation exposure.

Our earlier research led us to a working hypothesis that the early expression of IL1B is a key event in the initiation of the pulmonary response to radiation injury. Our current results continued to indicate that there is a significant increase in the early expression of IL1B, but not IL1A, in the lung at all dose levels within 1 h of irradiation. IL1B message levels remained elevated at the higher doses (>5 Gy) until 6 h postirradiation, then gradually declined, returning to base levels by 24 h. We also demonstrated a significant increase in the expression of the receptor IL1R2, but not IL1R1, that was again seen at all doses within 1 h of irradiation, with message levels remaining elevated at the higher doses at 6 h

postirradiation and returning to base levels by 24 h. Since IL1R2 has been shown to be functionally inactive (32,33) and a number of investigators have suggested that *IL1* regulation may occur partially through a receptor-ligand balance (34,35), we postulate that the IL1B-IL1R2 expression seen in this dose range may be part of a normal wound healing response and/or a tissue compensation mechanism.

The data also indicated a rapid increase in the level of expression of the chemokine receptors CCR1 and CXCR2. We considered this significant since the two receptors, in particular CXCR2, are associated with the regulation of neutrophils to sites of local inflammation (36). Indeed, a dose-dependent and temporally associated infiltration of neutrophils was seen in the lungs. This finding was significant because the murine pulmonary response to radiation has not been considered to be neutrophil driven. Although some investigators have suggested a critical role for the CXC chemokine receptor after other forms of lung injury (37–39), we believe that the transient nature of the neutrophil response and the temporal relationship with the observed apoptosis (compare Figs. 2 and 9) suggest that these events are part of a normal wound healing response.

Building upon earlier findings from our group that identified circulating markers that appeared predictive of late pulmonary injury (40,41), we analyzed plasma from animals at these early times, looking at a panel of proteins. Since the realization that circulating systemic markers could be used as a relatively simple assessment tool, clinicians and scientists alike have attempted to identify specific markers for a wide array of uses, including markers that may be predictive for radiation-induced late effects. This research has given rise to a number of circulating markers that are potentially predictive for radiation-induced lung effects, including transforming growth factor β (42,43), IL6 and IL1A (41). One of the proteins that we had identified in our earlier clinical study, IL6, demonstrated a highly significant and discriminatory increase after doses above 2 Gy at 6 h postirradiation (Figs. 4 and 5), although there was no corresponding change in IL6 mRNA expression in the lungs (additional analysis also demonstrated no change in liver tissue; data not shown). This contrasted with the changes in expression of keratinocyte chemoattractant, KC/CXCL1, which also showed a significant, dose-related increase in the plasma at 6 h postirradiation. KC/CXCL1, a neutrophil chemotactic factor, is a ligand for CXCR2 and has been shown to increase in expression after other pulmonary injuries (44); therefore, the CXCL1/CXCR2 relationship may be a key factor that is responsible for the increased recruitment of neutrophils into the lung (Fig. 9). However, since there is no current correlation between these early and transient increases in expression and the development of pulmonary late effects, such increases may only serve as surrogates of injury and not necessarily targets for mitigation. Overall, these findings were thought to support the potential use of IL6 and KC recognition in the circulation as part of a temporally related biodosimetry panel indicating radiation exposure; however, it is unclear whether their expression is specific to radiation injury rather than being part of a stress response. In addition, the differential response between pup and adult mouse lungs after radiation injury tempers enthusiasm for their use since the marker expression may relate to different end points in different segments of the population.

An important observation made as part of this study was the overall differential response between the adult and neonate animals. It has been demonstrated that, under many circumstances, children respond differently than adults to radiation injury, for example after radiation treatment (22). Since this differential is often exhibited as an apparent change in radiation sensitivity, it suggests that this population may be at altered or even increased risk of developing radiation-induced late effects. However, apart from case studies of overexposure in children, the majority of the available data in this population are limited to the monitoring that took place after the nuclear power accident at Chernobyl, which has

focused on the subsequent carcinogenesis and genomic changes (45). A small Ukrainian epidemiological study also suggested that children living around Chernobyl demonstrated higher rates of respiratory tract illness than those reported in the area prior to the incident (46).

This latter finding provides support for the contention that the pediatric lung may be an organ that is especially at risk from radiation injury since it has been shown by many investigators that children's lungs are highly susceptible to damage from exposure to many environmental toxicants (47–49). This is principally a result of the protracted maturation of the respiratory system, which in humans extends from the embryonic phase through to adolescence (50). Besides an immature respiratory system at birth, children possess unique differences in their physiology and behavioral characteristics compared to adults. These are believed to augment the vulnerability of their developing lungs to perturbations by environmental toxins. Indeed, our results demonstrated that the early changes in IL1B and IL1R2 expression that were observed in the adult animals after irradiation could not be detected in mouse pups until they had reached 28 days of age (Fig. 13b), which coincides with the time of weaning, although the detection of apoptosis in the lungs of all age groups (Fig. 10) indicated that pulmonary injury occurred to a similar extent. This finding is of concern since work from our group has previously shown that damage to the lung during the developmental period may act as a priming agent for a subsequent injury, triggering sensitization to a secondary stimulus (51), suggesting that factors that disrupt the developmental events may have significant long-term consequences.

Although rapid and similar increases in the level of expression of CXCL1 were seen in the plasma of both mouse pups and adults (Fig. 11), the increase was not coincident with neutrophil infiltration in the mouse pups; infiltration was seen only in the adult animals (Fig. 12). Therefore, our findings emphasize the need to recognize that radiation-induced late effects in young animals (and by extension, human children) may differ from adult responses and support the contention that current models do not sufficiently predict for late effects in a pediatric population (52). There is also an increased expression of proinflammatory cytokines within the pulmonary tissue in the TBI animals compared to the whole-lung-irradiated animals, despite the fact that the tissue itself would have received the same dose. This suggests a systemic influence on the local tissue response that could support an altered immune response. If true, it may be worth speculating that such an alteration may affect the lung's ability to respond to later challenges and that this may be exacerbated further in the pediatric population. Therefore, further study is required to assess whether any of these early proinflammatory markers play a role in the induction of late effects in this low-dose model and how age may affect not only the initial proinflammatory cytokine response but also the subsequent communications between injured parenchyma and infiltrating inflammatory cells; these studies are ongoing.

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FIG. 1.
Representative micrographs of TUNEL staining in C57BL/6J mouse lung after total-body irradiation with 1 Gy (panels a and b) and 10 Gy (panels c and d) at 1 and 6 h postirradiation, respectively.

FIG. 2. TUNEL-positive cells in C57BL/6J mice at 1, 6, 24 or 48 h postirradiation after 0–10 Gy administered to the whole lung (panel a) or as total-body irradiation (panel b). $n = 10$ per group. Error limits are standard errors.

FIG. 3.

Analysis of circulating KC/CXCL1 (panels a and b) and IL6 (panels c and d) after 0–10 Gy whole-lung and total-body irradiation, respectively, of C57BL/6J mice at 1, 6, 24 or 48 h. $n = 10$ per group.

FIG. 4.

Statistical analysis to assess the significance of cytokine expression, as measured by multiplex assay, in the plasma after whole-lung irradiation: plasma levels of IL6 at 6 h postirradiation (panel a) and KC/CXCL1 at 1 h (panel b) and 6 h (panels c and d) postirradiation, respectively. Error limits are standard errors.

FIG. 5.

Statistical analysis to assess the significance of the expression, as measured by multiplex assay, of the plasma levels of IL6 and KC/CXCL1 in C57BL/6J mice after whole-lung (panels a and d) or total-body irradiation (TBI) (panels b and c) at 6 h postirradiation. Error limits are standard errors.

FIG. 6.

Panel a: RNase protection assay measuring interleukin mRNA abundance in the lungs after whole-lung irradiation. IL1R2 and IL1 β are highlighted to aid in interpretation. Time course of changes in the relative mRNA abundance of (panel b) IL1A and IL1B and (panel c) IL1R1 and IL1R2 in lungs after irradiation. RNase protection assays were quantified by PhosphorImager analysis. All values were normalized to constitutively expressed mRNA encoding L32. Each bar represents the mean for a minimum of five mice. Error limits are standard errors.

FIG. 7.

Panel a: RNase protection assay measuring interleukin mRNA abundance in the lungs 1 h after total-body irradiation at 1 h. IL1R2 and IL1B are highlighted to aid in interpretation. Time course of changes in relative mRNA abundance of (panel b) IL1A and IL1B and (panel c) IL1R1 and IL1R2 in lungs after irradiation. RNase protection assays were quantified by PhosphorImager analysis. All values were normalized to constitutively expressed mRNA encoding L32. Each bar represents the mean for a minimum of five mice.

FIG. 8.

Time course of changes in relative mRNA abundance of CCR1 and CXCR2 after whole-lung irradiation. RNase protection assays were quantified by PhosphorImager analysis. All values were normalized to constitutively expressed mRNA encoding L32. Each bar represents the mean of a minimum of five mice.

FIG. 9. Number of neutrophils in the lungs of C57BL/6J mice after whole-lung irradiation. Error limits are standard errors.

FIG. 10. TUNEL-positive cells in mouse pups (4–14 days old) and adult (56 days old) C57BL/6J after total-body irradiation. Error limits are standard errors.

FIG. 11. Circulating KC/CXCL1 after 0 or 5 Gy total-body irradiation of mouse pups (4–14 days old) and adult (56 days old) at 6 h postirradiation. Error limits are standard errors.

FIG. 12. Number of neutrophils in the lungs of mouse pups (4–14 days old) and adults (56 days old) after 0 or 5 Gy total-lung irradiation. Error limits are standard errors.

FIG. 13.

Panel a: RNase protection assay measuring interleukin mRNA abundance in the lungs of adult (56 days old) mice and mouse pups 1 h after 0 or 5 Gy total-body irradiation. RNase protection assay: 10 μ g total lung RNA hybridized with 5.2×10^5 cpm for 18 h at 56°C. Panel b: Time course of changes in relative mRNA abundance of IL1A, IL1B, IL1R1 and IL1R2 in lungs of adult (56 days old) C57BL/6J mice and mouse pups at 1 h postirradiation. RNase protection assays were quantified by PhosphorImager analysis. All values were normalized to constitutively expressed mRNA encoding L32. Each bar represents the mean of a minimum of three mice.