Irradiation-Induced Pneumonitis Mediated by the CD95/CD95-Ligand System

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Pneumonitis is a dose-limiting side effect of radiotherapy. However, the underlying mechanisms of irradiation-induced pneumonitis are unclear. Several observations suggest that the CD95/CD95-ligand (CD95L) system is involved in this process. Therefore, we examined the development of pneumonitis in CD95- and CD95L-deficient and wildtype mice after single irradiation with 0 or 12.5 Gy by measuring breathing frequency, pulmonary resistance, and histopathologic changes. Although wildtype mice developed pathognomonic alterations characteristic of pneumonitis (judged by alveolar wall thickness, interstitial edema, and interstitial and peribronchial inflammation) that paralleled increased breathing frequency ratio on days 5–7 (*P < 0.03) with a maximum at day 37 (12.5 Gy, mean ratio = 1.05, 95% confidence interval [CI] = 1.01 to 1.08; *P = 0.04 versus 0 Gy, mean ratio = 0.997, 95% CI = 0.976 to 1.02; *P = 0.05) and pulmonary resistance (day 42, 12.5 Gy, mean = 0.51, 95% CI = 0.44 to 0.58 versus 0 Gy, mean = 0.40, 95% CI = 0.32 to 0.47; *P = 0.03) after irradiation, no such changes were detected in CD95- or CD95L-deficient mice. This report demonstrates for the first time, to our knowledge, that the CD95/CD95L system is important for the development of irradiation-induced pneumonitis. [J Natl Cancer Inst 2006;98:1248–51]

Pneumonitis is a dose-limiting side effect of total-body irradiation and is the main reason for dose restrictions during radiotherapy for any thorax-associated neoplasm. Pneumonitis is clinically associated with symptoms of respiratory failure and results in a mortality rate of up to 10% (1–3).

Currently, the mechanisms for irradiation-induced pneumonitis are still unclear. Although pneumonitis mostly occurs within the irradiated areas of the lung, it may spread to nonirradiated areas, indicating that humoral factors may be involved (4). The current working hypothesis suggests that complex alterations engaging lung epithelial cells (e.g., type 2 pneumocytes) (5), endothelial cells (6), and a perpetual cascade of cytokine expression patterns (7–9) are important for the induction of pneumonitis (10).

CD95 (also known as Fas) and CD95L (also known as FasL or CD178) are expressed on various cells and tissues, including the lung (e.g., bronchiolar and alveolar cells) (11). CD95 and CD95L are involved in the induction of apoptosis (12,13), proinflammatory cytokine responses (e.g., tumor necrosis factor-α, interleukin-8) (14,15), and the attraction of neutrophils (16,17). In this regard, it has been shown that acute lung injury induced by bacterial infection (18), bleomycin treatment (19), or intrapulmonary deposition of IgG immune complexes (20) may result in increased CD95 and CD95L expression and the induction of apoptosis and inflammatory responses, including secretion of defensins and/or cytokines. Moreover, the expression of CD95 and CD95L is increased after irradiation (21,22).

The present study was designed to define the contribution of CD95 and CD95L in the pathogenesis of irradiation-induced pneumonitis. Therefore, we assessed the development of pneumonitis after single irradiation (0 Gy [sham] or 12.5 Gy) of the right hemithorax in C57BL/6J mice with intact CD95 and CD95L (wild-type), CD95-deficient lpr mice, and CD95L-deficient gld mice with respect to physiologic (breathing frequency, pulmonary resistance, and pulmonary compliance) and histopathologic (cumulative inflammation score) changes. Four- to six-week-old female C57BL/6J wild-type (n = 67), CD95 receptor-deficient (lpr) (n = 53), or CD95L-deficient (gld) (n = 61) mice (Charles River laboratories, Sulzfeld, Germany) were housed (up to five mice per cage) in a standard barrier facility at room temperatures of 20–22 °C with a 12-hour light/dark cycle. Food and drinking water were provided ad libitum. Mice were subsequently enrolled to the study protocol with a body weight of approximately 20 g after adaptation to a totalbody plethysmograph for 14 days. All mouse protocols were approved by the University of Tuebingen animal protection board in conjunction with the regional council Tuebingen (Regierungssaediunim Tuebingen). Animal care was provided in accordance with the guidelines for care and use of laboratory animals (newest edition 25.05.1998 BGBl. I S. 1105; animal experiment R 3/01; R 1/04).

Mice were anesthetized with 2% isoflurane and placed in holders, and their bodies, excluding the right hemithorax, were shielded with 60 mm of lead. Mice were then irradiated with a single dose of 0 Gy (sham) or 12.5 Gy using a linear accelerator (dose rate = 4.7 Gy/min; n ≥ 5 mice per dose group).

After irradiation, breathing frequency was measured using a total-body plethysmograph with a chamber volume of 960 cm³. Mice were placed individually in the chamber, and pressure changes in the chamber were monitored, converted into an electric signal, which was filtered to remove interfering signals not caused by respiration, and amplified. The amplified signal was calibrated from 1 to 8 Hz (i.e., breaths per second) using an oscillator.

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See “Notes” following “References.”

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Breathing frequencies at rest were measured for 2 minutes and 40 seconds at the same time of day to minimize circadian changes. Breathing frequency was measured twice weekly for up to 30 weeks, and the breathing frequency ratio (breathing frequency at day x/breathing frequency at day 0) of sham-irradiated and irradiated mice was measured twice weekly from day 0 to 210 and fitted by a nonlinear regression model that allowed for a sinus function between days 5 and 70 after irradiation. Regression lines with 95% confidence intervals are shown from day 0 to 90 (details in Supplementary Data 1, available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue17). C57BL6/J mice: sham irradiated (black lines), decreased breathing frequency ratio over time (P<.001), no pneumonitic peak; irradiated (red lines), no change in breathing frequency over time, but a pneumonitic peak was observed (P<.03). GLD mice: sham irradiated (black lines), increased breathing frequency ratio over time (P<.001), no pneumonitic peak observed; irradiated (red lines), breathing frequency ratio increased over time (P<.001), no pneumonitic peak. LPR mice: sham irradiated (black lines), increased breathing frequency ratio over time (P<.001), no pneumonitic peak; irradiated (red lines), breathing frequency ratio increased over time (P<.001), no pneumonitic peak. A) The breathing frequency ratio (breathing frequency at day x/breathing frequency at day 0) of sham-irradiated and irradiated mice was measured twice weekly from day 0 to 210 and fitted by a nonlinear regression model that allowed for a sinus function between days 5 and 70 after irradiation. Regression lines with 95% confidence intervals are shown from day 0 to 90 (details in Supplementary Data 1, available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue17). B) Pulmonary resistance (Res) was measured in sham-irradiated mice (black bars) and irradiated mice (red bars) 42 days after irradiation. Pulmonary compliance was not altered in any mouse strain. P = .14 (C57BL6/J), P = .37 (LPR), P = .40 (GLD). Each series represents six independent experiments. Experimental details are provided in Supplementary Data 2 (available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue17). Pulmonary resistance and pulmonary compliance are expressed in absolute values. Means and upper 95% confidence intervals are shown. The number of independent experiments performed is indicated on each bar. P values (one-sided t test) were corrected for multiple comparisons according to the false-discovery rate procedure using the “R” statistical package.

In accordance with previously reported findings (5,19,25), lungs were analyzed at days 1, 21, 42, and 84 for the onset of the pneumonitic reaction and at day 210 to detect potential late effects. Histopathologic changes, i.e., alveolar wall thickness, interstitial edema, and interstitial and peribronchial inflammation, were judged by two independent investigators (T. Eldh, K. Nowak) in a blinded manner. For each of these morphologic alterations, a numerical score was determined. Scoring was assessed according to previously published scoring criteria (5,19,25) as follows: 0 = alterations in less than 10% of the fields viewed, 1 = in 10%–30%, 2 = in 30%–50%, 3 = in 50%–70%, and 4 = in more than 70%. A cumulative inflammation score was then determined for each group of mice (Supplementary Data 3, available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue17). Due to the development of lymphadenopathy in lpr and gld mice, these analyses were restricted to days 1–84 (26,27).

Determination of lung physiologic parameters revealed that in sham-irradiated wild-type mice, breathing frequency ratio decreased over time, whereas that of sham-irradiated lpr and gld mice increased, most probably due to lymphadenopathy, e.g., in the thorax, the mediastinum, and the bowel, which impairs normal breathing (26,27).
higher airway resistance than sham-irradiated mice at day 42 (day 42, 12.5 Gy, mean = 0.51, 95% CI = 0.44 to 0.58 versus 0 Gy, mean = 0.40, 95% CI = 0.32 to 0.47; *P* = .03; Fig. 1, B). Thus, in irradiated wild-type mice, at the time point of maximal increase in breathing frequency, a second physiologic parameter indicative of inflammatory lung injury was increased. In contrast, no differences were observed in irradiated and sham-irradiated gld (*P* = .349) and lpr (*P* = .349) mice (Fig. 1, B).

To further confirm the physiological significance of CD95 and CD95L in the development of irradiation-induced pneumonitis, we histologically examined the lungs of wild-type, lpr, and gld mice for characteristic pathognomonic alterations of pneumonitis. In sham-irradiated wild-type mice, no morphologic changes in lung tissue were detected throughout the observation period (days 1–84). In contrast, following 12.5-Gy exposure, a noteworthy augmentation in alveolar wall thickness, occurrence of interstitial edema, and interstitial and peribronchial inflammation in irradiated right lungs of wild-type mice was detected (Fig. 2, A) resulting in increased inflammation scores on days 21, 42, and 84, compared with sham-irradiated mice (Fig. 2, B). Moreover, a less pronounced yet clearly detectable inflammatory reaction was observed in the lead-shielded left lungs supporting the interpretation that humoral factors, like inflammatory cytokines or chemokines, might be involved in this form of secondary lung injury (Fig. 2, A and B).

In strong contrast to wild-type mice, the histopathologic analyses revealed no signs of a pulmonary inflammatory response in gld mice. Irradiated right lungs of gld mice remained undistinguishable from nonirradiated or shielded left lungs at all time points (Fig. 2, A).

A slight but statistically nonsignificant inflammatory response was observed in the right lungs of lpr mice at days 21 and 42 upon 12.5-Gy exposure when compared with sham-irradiated lpr mice (Fig. 2, A and B). However, the difference in the respective inflammation score was clearly less pronounced in lpr mice (approximately 2 points above nonirradiated littermates) compared with wild-type mice (approximately 5 points above nonirradiated littermates; *P* < .001) (Fig. 2, C). Similarly, no statistically significant histologic differences were observed in the shielded left lungs of mice treated with 0 and 12.5 Gy (Fig. 2, A and B).

In conclusion, our data demonstrate a central role of CD95 and CD95L in the development of irradiation-induced pneumonitis. In response to hemithoracic irradiation, a statistically significant increase in breathing frequency ratio and pulmonary resistance accompanied by histologic signs of pulmonary inflammation was only detected in wild-type mice. This pneumonitic reaction was considerably diminished in lpr mice and completely absent in gld mice. These data are consistent with recent findings of reduced lung injury induced in gld and lpr mice compared with wild-type mice by intrapulmonary deposition of IgG immune complexes (20).

However, it has to be taken into account that the time curve and severity of a given pneumonitic response in mice is strain-dependent. Thus, a potential limitation of our study is the fact that all conclusions are based on the use of C57BL6/J mice. Nevertheless, our findings identify CD95 and CD95L as potential therapeutic targets in irradiation-induced pneumonitis. Future studies will define whether pharmacologic inhibition of CD95 and
CD95L might be suitable for the prevention or treatment of the inflammatory response to oncologic irradiation therapy.

REFERENCES


NOTES

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