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CHANGES IN EXPRESSION OF INJURY AFTER IRRADIATION OF INCREASING VOLUMES IN RAT LUNG

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Purpose: To improve the cure rates of thoracic malignancies by radiation dose escalation, very accurate insight is required in the dose delivery parameters that maximally spare normal lung function. Radiation-induced lung complications are classically divided into an early pneumonitic and a late fibrotic phase. This study investigated the relative dose–volume sensitivity, underlying pathologic findings, and consequentiality of early to late pathologic features.

Methods and Materials: We used high-precision, graded dose–volume lung irradiations and followed the time dependency of the morphologic sequelae in relation to overall respiratory function.

Results: Two distinct pathologic lesions were identified in the early postirradiation period (6–12 weeks): vascular inflammation and parenchymal inflammation. Vascular inflammation occurred at single doses as low as 9 Gy. This translated into early respiratory dysfunction only when a large lung volume had been irradiated and was reversible with time. Parenchymal inflammation was seen after higher doses only (onset at 16 Gy), progressed into later fibrotic remodeling but did not translate into dysfunction at a 25% lung volume even after single doses up to 36 Gy.

Conclusion: Our data imply that a low dose scattered over a large lung volume causes more early toxicity than an extreme dose confined to a small volume. Such findings are crucial for clinical treatment planning of dose escalations and choices for modern radiotherapy techniques. © 2007 Elsevier Inc.

INTRODUCTION

The inclusion of healthy lung tissue into the radiation field is inevitable during radiotherapy (RT) for thoracic tumors. Because the resulting pulmonary toxicity may be life-threatening, efforts have been aimed at finding reliable measures that would predict whether a treatment plan is safe for an individual patient. Modern three-dimensional treatment planning techniques yield accurate dosimetric data throughout a patient’s lung, enabling investigations of the relation between toxicity and various dose–volume parameters. Several simple parameters (e.g., mean lung dose, volume irradiated to >20 or ≥30 Gy) have been related to the development of symptomatic RT-induced lung injury and models have been created that relate the dose–volume histogram to the normal tissue complication probability (NTCP) (1, 2).

Nevertheless, if used alone, none of these parameters offered a predictive power sufficient for clinical decision making (3). It is generally agreed that multiparameter predictive models are necessary for clinical use. Information on the pre-RT overall lung function (3, 4), inhomogeneous lung perfusion (3, 5), upper or lower lung tumor location (6, 7), and cytokine expression (8) have all been shown to improve the prediction if used in conjunction with the dose–volume parameters. However, even those models cannot separate patients who develop or stay free of respiratory symptoms with sufficient accuracy. Thus, the optimal combination of predictive parameters remains unknown. This has seriously hampered efforts to improve cancer cure rates by escalating the radiation dose to a tumor.

Experimental studies have allowed testing of a better defined set of different treatment conditions than is achiev-

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able in clinical practice. Such studies have led to the concepts of dose–volume interactions and regional differences in pulmonary radiosensitivity (9–12). Also, experimental studies have allowed detailed investigations on pathophys- iologic mechanisms that can help to improve the formulation of predictive models and offer explanations for the enduring contradictory observations found in published re- ports. Radiation-induced lung injury has been traditionally divided into an early inflammatory phase, termed “radiation pneumonitis,” and a late fibroproductive phase, termed “fibrosis in humans” (13–15) and “fibrosis in animals” (16, 17). Dispute exists as to whether the two entities are a cascade of consequential and sequential processes, albeit involving multiple cell types (18, 19) or whether they are independent events (2, 16, 20).

In the present study, we addressed these latter questions and investigated the morphologic abnormalities emerging at different times after RT at three different volume levels (100%, 50%, and 25%) in rat lung and how they translated into impairment of overall lung function. Phases with distinct morphologic and functional characteristics were defined within the experimental follow-up. It was found that the character of the morphologic lesions in the early phase differed qualitatively according to the dose administered and that this was critical for the development (or nondevelopment) of the consequential late damage. Furthermore, the volume irradiated had a marked influence on the probability of each type of morphologic damage to translate into respiratory dysfunction. Clearly, the responses to irradiation of a small volume to a high dose or of a large volume to a low dose followed different rules with regard to their impact on overall lung function at increasing lengths of times after the exposure.

METHODS AND MATERIALS

Animals

Adult male albino Wistar rats of the Hsd/Cpb:WU strain bred in a specific pathogen-free colony (Harlan-CPB, Rijswijk, The Netherlands) were used in these experiments. They were housed five to a cage under a 12-h light/12-h dark cycle and fed rodent chow (RMH-B, Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were performed in agreement with the Netherlands Experiments on Animals Act (1977) and the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Strasbourg, 18.III.1986).

Collimator design and irradiation procedure

On the basis of thoracic computed tomography scans of 5 healthy rats weighing 300–340 g, the borders of the collimators were calculated to expose either 100% (whole lung plus margin), 50% ± 5%, or 25% ± 5% of the total lung volume, including the alveolar parenchyma and bronchial structures encompassed within. Three-millimeter lead collimators were then constructed. Details of the procedure have been previously published (21, 22). Simulator images of the five resulting irradiation portals are shown in Fig. 1a–e. The alveoli essential for the gas exchange were not homogeneously distributed over the whole lung volume, because perihilar regions contained more large bronchi than did the peripher al regions (Fig. 1f). A similar finding was earlier shown in mice (11). The two 50%-volume irradiation cohorts represented two distinct cases. Almost the entire right lung was irradiated in the 50% right cohort (Fig. 1b). The irradiation field thus encompassed not only the alveolar parenchyma, but also the large bronchi and vessels branching from the right hilum, reducing the affected volume of alveolar tissue (decisive for the functional response) to slightly <50%. This cohort was therefore labeled “50%min.” The 50% lateral cohort was irradiated to the lateral parts of both lungs that consisted exclusively of alveolar tissue (Fig. 1c). Therefore, the volume of affected alveolar tissue in this case was truly 50% of total lung volume and was even >50% of total alveolar tissue volume. For this reason, the cohort was labeled “50%plus.”

The volume of the heart in the irradiation fields was 99% ± 1% for the whole lung irradiation, 25% ± 5% for the 50% right and 25% apex irradiations, 3% ± 3% for the 50% lateral irradiation, and 1% ± 1% for the 25% base irradiation. However, the radiation doses used in the whole lung irradiation were less than the threshold, estimated to be 18–19 Gy, for early subclinical cardiac injury (23). Irradiation of 25% of the heart volume has previously been shown not to influence global respiratory function as measured by a breathing rate assay (9). Thus, the extent of heart irradiation in the present set up was considered insignificant.

Details of the irradiation procedure and dosimetry have been previously published (9). In brief, the total doses were delivered using a 200-kV X-ray source from two opposing anteroposterior and posteroanterior fields. The nominal dose rate was 2.68–2.90 Gy/min. The dose was determined by the dose–time per field product (24). The calculated dose at the lung surface varied from 10 Gy for the whole lung irradiation to 3.88 Gy for the 50% right irradiation. A breathing rate assay (9) was used to study the effects of the irradiation on the heart. The heart was defined as the volume of the heart in the irradiation fields was 99% ± 1% for the whole lung irradiation, 25% ± 5% for the 50% right and 25% apex irradiations, 3% ± 3% for the 50% lateral irradiation, and 1% ± 1% for the 25% base irradiation. However, the radiation doses used in the whole lung irradiation were less than the threshold, estimated to be 18–19 Gy, for early subclinical cardiac injury (23). Irradiation of 25% of the heart volume has previously been shown not to influence global respiratory function as measured by a breathing rate assay (9). Thus, the extent of heart irradiation in the present set up was considered insignificant.
anesthetized and underwent sham irradiation. The control animals were chosen according to the published data (22, 24–26) and on small, pilot feasibility experiments. Care was taken to avoid lethal irradiation using a whole-body plethysmograph. Details of the procedure have been previously published (9). Serial sections (3 μm) containing standardized samples of every irradiated lobe were stained with hematoxylin and eosin or Masson’s trichrome stain for collagen fibers (blue-green) and examined by light microscopy. In the entire tissue cross-section on each slide, blinded scoring of the vascular changes and parenchymal changes (as described in the “Results” section) was done separately using two semiquantitative scoring scales. The vascular changes were scored using a 0–3-point scale, with 0 indicating 0–5, 1 indicating 6–12, 2 indicating 13–20, and 3 indicating >20 affected vessels with a diameter >300 μm—or double these amounts for vessels ≤300 μm. The scoring system for parenchymal injury was based on the size of the inflammatory or fibrotic focal lesions on each slide: no foci present, score 0; small foci present (focus <0.5 of 100× magnification field), score 1; medium foci present (focus volume 100× field), score 2; large foci present (focus volume 40× field) and total affected area volume 50% of total tissue cross-section, score 3; and confluent foci present (focus exceeded 40× field) and total affected area >50% of total tissue cross-section, score 4. One hematoxylin and eosin and one Masson’s trichrome slide per rat were examined and assigned a joint score for vascular damage (VD) and a joint score for parenchymal damage. See Table 2 for a simplified version of the scores.

Follow-up and morbidity

A total of 194 animals (including 14 controls) were involved in the study and were inspected a minimum of twice a week for general health and were weighed biweekly. The rats continued to grow, but the 11–12-Gy dose groups of the 100% volume cohort and the 18–22-Gy dose groups of the 50% volume cohort lagged behind the controls by ≤12% in their weight gain. The only extrapulmonary toxicity encountered was moist skin desquamation in the irradiation fields of animals irradiated to doses of ≥20 Gy. It was successfully managed by topical application of gentian violet. The number of animals per dose group decreased during the follow-up period because of regular killing of the rats for histologic examination. The number of rats per group was 9, 7, and 5 at 0–8, 10–26, and 28–38 weeks, respectively. The control group consisted of 14, 10, and 7 animals in these periods.

The different volume cohorts were irradiated 2–3 months apart. Because of the 9-month follow-up period, they overlapped in time and were housed in the same room of the animal laboratory. Several control animals were sham irradiated and then followed up with each cohort. We also performed an interexperiment comparison between the first and last cohort that confirmed the reproducibility of the data.

Breathing rate assay

The breathing rate (BR) at rest was recorded for each rat at <1 week before irradiation and then every 2 weeks to 38 weeks after irradiation using a whole-body plethysmograph. Details of the procedure have been previously published (9). The mean BR for each dose group in breaths per minute was calculated at each point, as well as for Weeks 6–12, 16–28, and 34–38. Those three intervals encompassed three distinct periods of BR dynamics, early, intermediate, and late, on the basis of differences in lung function as described previously (9). Also, an individual mean BR was calculated for each animal in those three periods. The ordinal BR data were converted into quantal BR data by applying a rule. If the individual mean BR in a given period exceeded a cutoff value, defined as the mean BR of the age-matched control group in that period ± 2 SD, the rat was considered a responder (meeting the nominal level of statistical significance of 0.05). The cutoff value was 183, 180, and 188 breaths per minute in the early, intermediate, and late periods, respectively.

Histologic examinations

Two rats per dose group and three control rats were killed at random for morphologic examination of their lung tissue at 8, 26, and 38 weeks after irradiation. Details of the procedure have been previously published (9). Serial sections (3 μm) containing standardized samples of every irradiated lobe were stained with hematoxylin and eosin or Masson’s trichrome stain for collagen fibers (blue-green) and examined by light microscopy. In the entire tissue cross-section on each slide, blinded scoring of the vascular changes and parenchymal changes (as described in the “Results” section) was done separately using two semiquantitative scoring scales. The vascular changes were scored using a 0–3-point scale, with 0 indicating 0–5, 1 indicating 6–12, 2 indicating 13–20, and 3 indicating >20 affected vessels with a diameter >300 μm—or double these amounts for vessels ≤300 μm. The scoring system for parenchymal injury was based on the size of the inflammatory or fibrotic focal lesions on each slide: no foci present, score 0; small foci present (focus <0.5 of 100× magnification field), score 1; medium foci present (focus volume 100× field), score 2; large foci present (focus volume 40× field) and total affected area volume 50% of total tissue cross-section, score 3; and confluent foci present (focus exceeded 40× field) and total affected area >50% of total tissue cross-section, score 4. One hematoxylin and eosin and one Masson’s trichrome slide per rat were examined and assigned a joint score for vascular damage (VD) and a joint score for parenchymal damage. See Table 2 for a simplified version of the scores.

Statistical analysis

The dose dependency of the BR response was evaluated by calculating the significance of Pearson’s correlation coefficient from scatter plots of the data. The levels of morphologic and functional responses after irradiation of a 25% volume in the lung apex or lung base were compared using the nonparametric Mann-Whitney U test. The nominal level of statistical significance was 5%.

The quantal BR data were analyzed using a probit model. This yielded sigmoid dose–response curves representing estimates of NTCP and estimates of the dose inducing the response in 50% of the irradiated animals (ED50) during each of the three follow-up periods. The ED50 values with their 95% confidence intervals allowed the comparison of the functional response of the irradiated volumes.

RESULTS

Whole lung irradiation (100% volume, dose range 9–12 Gy) resulted in alterations in both pulmonary morphology and function within the first 3 months after irradiation. Morphologically, at 8 weeks, the prominent finding was edema of vascular walls and perivascular mononuclear infiltrates affecting both large and small vessels (Fig. 2d,g). This inflammatory VD had disappeared at Weeks 26 (Fig. 2e,h) and 38 (Fig. 2f,i), although a variable degree of vascular sclerosis persisted (Fig. 2e,f,h). In contrast to the prominent VD, the changes within the alveolar parenchyma, whether inflammatory or fibrotic, were rare, and, if found, minimal at all points. Respiratory function, as represented by BR, showed dose-dependent (p <0.01) impairment between 4 and 14 weeks but had returned to normal at 16 weeks and continued so for the rest of the follow-up period (Fig. 3a). Thus, this low-dose/large-volume irradiation resulted in marked functional deficit during the early phase
but induced almost no alterations in the later periods (Fig. 4a,c,e). This suggested that the threshold for the early injury occurring after this type of irradiation was lower than the threshold for the chronic injury occurring after this type of irradiation, as evidenced by the NTCP curves (Fig. 4b,d,f) and the dose required to obtain a 50% effect (ED$_{50}$ values, Table 1).

The effects of the 50% volume irradiation (dose range,
16–22 Gy) have been previously described (9). Only the two 50% volume cohorts that did not entail significant cardiac irradiation were included in the present study to exclude the indirect effects on pulmonary function (9, 12). Those cohorts also represented irradiation of slightly different volumes of alveolar tissue because of the varying proportion of bronchi (see Fig. 1 and the “Methods and Materials” section). Three phases of response were distinguished: an early phase (Weeks 6–12) of focal exudative inflammation in the lung parenchyma paralleled by the first BR peak (Fig. 4a); an intermediate phase (Weeks 16–28), and a late phase (Weeks 34–38), the latter two both characterized by fibroproductive inflammation with either an increase in BR (intermediate phase, Fig. 4c) or a variable BR recovery (late phase, Fig. 4e). Although the intraparenchymal inflammatory foci dominated the morphology findings at 8 weeks, the VD was also present, both inside the foci and around them. The NTCP curves for each period are shown in Fig. 4b,d,f, and the ED$_{50}$ estimates are listed in Table 1. The expression of symptoms in the early and late periods was stronger in the 50%plus cohort but the 50%min irradiation was tolerated better (Fig. 4a,b,e,f). This subtle change in volume did not matter in the intermediate period when both 50% irradiation groups experienced severe functional detriment (Fig. 4c,d). Furthermore, almost all the early symptoms (100% and 93% in the 50%min and 50%plus cohorts, respectively) led to morbidity in the later phases. In addition, some symptoms (58% and 22% in the 50%min and 50%plus cohorts, respectively) arose de novo in the intermediate phase, hallmarked by the onset of fibrosis. Thus, the threshold for symptoms of late fibrosis appeared lower than that for symptoms of early pneumonitis after intermediate-dose/intermediate-volume irradiation.

The 25% volume irradiation (dose range, 27–36 Gy), aimed at either the right lung apex or right lung base, led to the formation of inflammatory foci in the irradiated parenchyma 8 weeks after irradiation. Just as after the 50% irradiation, the foci were marked by mononuclear infiltration of alveolar spaces and interstitial edema, but collagen deposition was already more pronounced at this early point (Fig. 2). This progressed into confluent fibrosis at Weeks 26 and 38 when the interstitium was expanded by collagen and mononuclear infiltration obliterated the alveolar spaces (Fig. 2k,l). Also, signs of VD were present as vascular wall edema in the early period (not shown) and vascular hyper trophy and sclerosis at the intermediate and late periods (Fig. 2k,l). Functionally, the 25% volume irradiation induced moderate BR increases in the early and intermediate periods with generalized recovery after 28 weeks (Figs. 3b,c and 4a,c,e). Dose dependency was neither expressed morphologically nor functionally, indicating that a maximal effect had already been achieved at (or less than) the lowest dose of 27 Gy. Differences in the level of response between the apex and base were not observed morphologically and were nonsignificant in function ($p_{early} = 0.09$, $p_{interm} = 0.3$, and $p_{late} = 0.06$). The NTCP curves are shown in Fig. 4b,d,f, and the ED$_{50}$ estimates are listed in Table 1. As with after the 50% volume irradiation, most of the early symptoms (71%) were followed by intermediate symptoms, but 33% of the latter arose de novo. However, the level of respiratory dysfunction after this high-dose/small-volume irradiation was never as severe as after the 50% volume irradiation. This confirms that the effect on global function is very volume dependent.

The patterns of responses for all volumes irradiated are summarized in Table 2. In the 100% volume cohort, VD was the only morphologic lesion observed across the applied dose range and thus had to be responsible for the functional response noted in the early phase (Fig. 4a,b). In the case of the two 50% volume cohorts (50%min and 50%plus), the vascular changes were widespread after 16 Gy and the inflammatory (later fibrotic) parenchymal foci were sparse and small. Because little functional deficit was detected in the 16-Gy dose groups (Fig. 4b,d,f), it can be deduced that neither the VD spread over the $\pm$50% lung
volume nor the mild parenchymal injury were sufficient to influence function. Larger doses (≥18 Gy) given to the 50% volume, however, led to substantial parenchymal injury with functional consequences in all phases of follow-up for the 50% plus cohort and, to a lesser extent, for the 50% min cohort (Fig. 4b,d,f). Although the VD was also present, the morphologic and functional outcomes were clearly dominated by the parenchymal injury. The same applied to the morphologic picture in the 25% volume cohorts, and, yet, the functional outcome was completely different, marked by good tolerance throughout the follow-up period.

The respiratory symptoms in the early phase were linked to the VD resulting from low-dose/large-volume irradiation and to acute parenchymal inflammation resulting from intermediate-dose/intermediate-volume irradiation. During the intermediate phase, functional loss occurred after intermediate-dose/intermediate-volume irradiation that led to a blending of acute inflammation with progressive fibrosis. Late phase functional injury resulted from confluent fibrosis propagated across the 50% plus volume. Fibrotic remodeling limited to 50% of alveolar volume, regardless of the radiation dose used, was without any consequences for function at those late points.

### DISCUSSION

To develop clinically reliable NTCP models for pulmonary RT, a thorough knowledge of how the dose–volume parameters interact with the responding tissue and its biology is necessary. In this study, we investigated the dose–volume relationship for pulmonary RT...
Rubin and Casarett (15) suggested that injury to the pulmonary vasculature played a primary role in the early radiation response and that mild damage could subside with no chronic reaction. Furthermore, early changes in lung perfusion showed a tendency for recovery with time in patients receiving fractionated lung doses of ≤50 Gy (31) compared with a continuous progression in patients irradiated to >50 Gy (32). Similarly, early radiographic pulmonary changes in breast cancer patients, in whom the lung dose is typically <50 Gy, have shown complete resolution (19, 33) in contrast to reports of chronic fibrotic scarring seen in most patients after thoracic RT (13, 14, 30). Even in rats, an early reduction in pulmonary blood flow returned to normal after single doses of ≤13.5 Gy, but higher doses (≥18 Gy) led to a second reduction at 16 weeks (34). All these findings suggest that milder VD is reparable but more severe parenchymal injury progresses to fibrosis. If, hypothetically, our single-dose regimens were translated into fractionated treatment using the linear-quadratic model (α/β = 3.3 Gy), the VD would have already occurred after 25 fractions of 1 Gy—a dose commonly delivered to the lung during photon beam therapy for breast cancer. The disappearance of sequelae with the passing of the acute phase after such treatment (19, 33) would match the occurrence of VD. In contrast, the potential of the early VD to cause serious hemodynamic problems, if spread over a large volume, should not be underestimated. In particular, during dose-intensified IMRT, low-to-intermediate doses can be delivered to significant portions of healthy lung and yield unexpected toxicity (35, 36). This underscores the need for investigations on the dose–volume thresholds for clinically relevant VD in humans.

When a higher dose producing the parenchymal injury was given, a smaller irradiated volume sufficed to cause a

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Table 2. Relation between morphologic and functional findings after irradiation of varying volumes in rat lung

<table>
<thead>
<tr>
<th>Period</th>
<th>End point</th>
<th>100% (9–12 Gy)</th>
<th>50%min (16 Gy)</th>
<th>50%min (18–22 Gy)</th>
<th>50%plus (16 Gy)</th>
<th>50%plus (18–22 Gy)</th>
<th>25% (27–36 Gy)</th>
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<td>+</td>
<td>+</td>
<td>NSc</td>
<td>NSc</td>
<td>+</td>
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<tr>
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<td>+/-</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
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<td>0</td>
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<td>0</td>
<td>+/-</td>
<td>+</td>
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<td>+/-</td>
<td>+</td>
<td>NSc</td>
<td>NSc</td>
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<tr>
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<td>+</td>
<td>+</td>
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</table>

**Abbreviations:** VD = vascular damage; H/S = hypertrophy and sclerosis of vascular walls; NSc = not scored; other abbreviations as in Table 1.

Semi-quantitative scale for morphologic and functional findings: 0, lesion absent or no responders; +/- lesion mostly absent or few individual responders; +, lesion present or <50% of responders; ++, lesion widespread or >50% of responders.

volume–time dependency of the morphologic sequelae of lung irradiation and its relation to overall lung function. We identified two distinct pathogenetic processes appearing in the early time window of radiation pneumonitis and having an unequal impact on later pathologic features. The VD induced by lower doses showed a large capacity for recovery with minimal structural or functional consequences >4 months after irradiation. However, the parenchymal inflammation produced by higher doses almost invariably ended in morphologically and clinically expressed fibrosis. Furthermore, the irradiated volume determined whether a specific type of morphologic damage resulted in functional loss. Large-volume irradiation produced the VD with early functional loss at low doses, but small-volume irradiation did not lead to substantial function loss at any postirradiation interval despite the very high doses and severe parenchymal injury. Our findings may facilitate risk prediction of dose-escalation treatments and intensity-modulated radiotherapy (IMRT) in lung cancer patients for which both high-dose and low-dose exposures are involved.

To date, risk-predictive models have considered pneumonitis as one pathogenetic unit with uniform dose–volume–response parameters (27). We have shown that although the “classic” parenchymal inflammation required a large dose and an intermediate irradiated volume to be expressed as an early functional deficit, the damage to the pulmonary vascular bed was elicited by doses far less than the threshold for the parenchymal injury but needed to involve larger volumes to influence lung function. Moreover, the VD, unlike the parenchymal inflammation, had largely receded by 4 months after exposure. Several observations published previously support our findings. Two types of damage, endothelial and epithelial, have been previously described to occur in the early phase of pulmonary radiation injury (28–30).
functional response. In our experiments, the cutoff was about 50% of the total lung volume, because the 50% plus volume cohort showed functional deficits consistently throughout the follow-up period, but in the 50% minus cohort, the impairment was seen only in the intermediate phase. It seemed that the pulmonary reserve capacity during the early and late periods was just sufficient when slightly <50% of alveolar tissue had been affected (the 50% minus volume vs. the 50% plus volume), suggesting a very steep volume effect for tolerance at this volume interval. At lower volumes (25% volume irradiation), even extreme doses (highest single dose used in this study was a biologic equivalent of 267 Gy in 2-Gy fractions, α/β = 3.3 GY), which induced severe parenchymal inflammation and fibrosis, had only a minor impact on respiratory function. This argues against the propriety of risk prediction using dose-averaging approaches such as the mean lung dose (5, 37). In contrast, it supports the clinical findings that a safe volume to irradiate with high doses is probably <25% (38, 39). In patient populations in which the pre-RT respiratory function is often compromised by pre-existing pulmonary morbidity, limits for caution would definitely be more stringent than in our experiment. Nevertheless, the results can be summarized in a dictum: “a lot to a little is tolerated better than a little to a lot.” It would be of interest to assess in future studies how the development of the VD vs. the parenchymal inflammation/fibrosis may be influenced by a prophylactic or therapeutic application of anti-inflammatory agents (40).

CONCLUSION

We have reported for the first time how two distinct forms of postirradiation morphologic damage have different implications for tolerance after irradiation of small or large volumes in the rat lung. The inflammatory VD occurring at low doses affected lung function when a large volume was irradiated. This could have implications for treatment techniques in which the dose is spread over a large volume, such as with IMRT. The parenchymal injury occurred at higher doses but was already able to compromise function at intermediate irradiated volumes. Nevertheless, the functional consequences after irradiation of small volumes were minimal owing to the reserve capacity of the lung. This indicates that dose escalation to very high doses, but involving small lung volumes, is a feasible therapeutic strategy, provided the larger lung volumes are protected from low-to-intermediate dose exposure. Such findings may be used in designing clinical trials that study NTCP prediction after novel RT techniques for lung cancer such as IMRT or proton RT.

REFERENCES


