RADIATION RESEARCH **191**, 271–277 (2019)
0033-7587/19 $15.00

DOI: 10.1667/RR15182.1

Impact of Radiotherapy Concurrent with Anti-PD-1 Therapy on the Lung Tissue of Tumor-Bearing Mice

Feng Wang,[a,b] Yuan Luo,[a,b] Xiaoli Tian,[a,b] Shijing Ma,[a,b] Yingming Sun,[a,b] Chengcheng You,[a,b] Yan Gong[c,1] and Conghua Xie[a,b,1]

[a] *Department of Radiation and Medical Oncology,* [b] *Hubei Key Laboratory of Tumor Biological Behaviors, Hubei Cancer Clinical Study Center and* [c] *Department of Biological Repositories, Zhongnan Hospital of Wuhan University, Wuhan, China*

Wang, F., Luo, Y., Tian, X., Ma, S., Sun, Y., You, C., Gong, Y. and Xie, C. Impact of Radiotherapy Concurrent with Anti-PD-1 Therapy on the Lung Tissue of Tumor-Bearing Mice. ***Radiat. Res.*** **191, 271–277 (2019).**

Pneumonitis is a common adverse effect found in non-small cell lung cancer patients after radiotherapy or immune checkpoint inhibitor treatment. We investigated the effects of these two therapies, combined, in the lung tissue of an orthotopic tumor-bearing mouse model. The mice received an 8 Gy dose three times with or without 200 μg anti-programmed death-1 (anti-PD-1) antibody intraperitoneal injection every three days. Lung tissues were H&E stained to determine histological changes. The serum levels of cytokines, such as interferon-γ, tumor necrosis factor and interleukin-5, were detected by cytometric bead array. The neutrophil infiltration was evaluated by immunohistochemical staining for myeloperoxidase. The lung injury score was higher in the treated groups than the control group, especially in the combined treatment group, in which the proportion of neutrophils in lung tissues was significantly higher compared to any other groups. Similarly, the CD4/CD8 ratio of the lung tissues in the combined treatment group, as well as the serum levels of interferon-γ, tumor necrosis factor and interleukin-5, were significantly higher than the other groups. These findings indicate that radiation combined with anti-PD-1 treatment leads to more severe lung injury in the orthotopic tumor-bearing mouse model, accompanied by increased neutrophil infiltration and increased inflammatory response.

INTRODUCTION

Radiotherapy is the standard approach to treat lung cancer and is widely used for early-stage non-small cell lung cancer (NSCLC), locally advanced NSCLC and small cell lung cancer (SCLC). The effect of stereotactic radiotherapy on NSCLC patients, who were not suitable for surgery at the early stage, was comparable to that of surgery (*1*). In addition, the effectiveness of prophylactic brain irradiation in the limited stage of SCLC (*2*) was a major breakthrough discovered in recent years. However, long-term treatment outcomes suggest that radiotherapy has benefits as well as side effects, it is beneficial for tumor regression, while also affecting nearby normal tissues (*3, 4*). Radiation-induced lung injury (RILI) is one of the most serious and common side effects in lung cancer patients receiving radiotherapy (*4*), with an RILI incidence reported to be 5–20% (*5, 6*). Radiation-induced pneumonitis and pulmonary fibrosis are two major events that occur with the development of RILI (*6*). Pneumonitis is an acute inflammatory response, which is usually observed 1–6 months after radiotherapy, and subsequent radiation-induced lung fibrosis (RILF) is considered to be an irreversible adverse event in the later stages of RILI (*7*). Previous studies indicate that radiotherapy leads to an imbalance in the immune system, which contributes significantly to RILI development (*8*).

Programmed death-1(PD-1) is an immunoglobulin superfamily stimulator molecule, mainly expressed on the T-cell surface (*9*). It has two ligands, programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) (*10*). Compared to normal tissue cells, a variety of tumor cells have higher expression levels of PD-L1, which is highly correlated with low reactivity of tumor to the immune system. The highly expressed PD-L1 impairs T-cell immune killing, thereby contributing to the immune escape of tumor cells. PD-L1 was reported to be negatively correlated with prognosis (*11*). These biological characteristics make PD-1 and PD-L1 novel targets for tumor therapy. Nivolumab and pembrolizumab were approved by the U.S. Food and Drug Administration to treat malignant tumors, including melanoma (*12*), NSCLC (*13*) and renal cell carcinoma (*14*). The current clinical trials of immune checkpoint inhibitors for NSCLC have shown that most of the adverse events, including vomiting and gastrointestinal response, were less frequent than from chemotherapy, while other adverse

Editor's note. The online version of this article (DOI: 10.1667/RR15182.1) contains supplementary information that is available to all authorized users.

[1] Address for correspondence: Department of Radiation and Medical Oncology and Biological Repositories, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuhan, Hubei 430071, China; email: chxie_65@whu.edu.cn or yan.gong@whu.edu.cn.

events, including thyroid-related symptoms and immune pneumonitis, were more frequent (*15*). The incidence of pneumonitis in patients with PD-1 blockade treatment was approximately 3–7%, which was significantly higher than in the control group (*16*).

Both radiotherapy and the immune checkpoint inhibitors may result in immune system imbalance (*17, 18*). There are also a variety of clinical trials that explore the efficiency of radiotherapy combined with immune checkpoint inhibitor treatment in NSCLC patients (Table 1). Therefore, it is essential to understand the interaction between radiotherapy, immune checkpoint inhibitor treatment and the immune system in lung tissues.

A published retrospective analysis of the KENOTE-001 test showed that the incidence of pneumonitis in patients who received radiotherapy before the immunosuppressive point inhibitor (63%) was higher than in patients who had not previously received radiotherapy (40%) (*19*). It suggested that radiotherapy and immune checkpoint inhibitors might have synergistic effects on the occurrence of pneumonitis. While there are retrospective studies on the effects of radiotherapy and immune checkpoint inhibitors on the development, metastasis and invasion of tumors, the combination effects of these two therapies on lung tissue requires additional studies. Therefore, we established an orthotopic tumor-bearing mouse model to simulate a tumor environment in mice and to explore the effects of combined treatment using radiation and immune checkpoint inhibitor administration in mouse lung tissues.

TABLE 1
Clinical Trials of Radiotherapy and Immune Checkpoint Inhibitor in NSCLC Patients

Clinical Trial No.	Stage of NSCLC	Mode	Immune checkpont inhibitors
NCT03158883	Advanced	SABR	Avelumab (anti-PD-L1)
NCT03245177	Advanced	RT	Pembrolizumab (anti-PD-1)
NCT03044626	Advanced	RT	Nivolumab (anti-PD-1)
NCT03307759	Advanced	SABR	Pembrolizumab (anti-PD-1)
NCT02434081	III Stage	RT	Nivolumab (anti-PD-1)
NCT03050554	Early stage	SBRT	Avelumab (anti-PD-L1)
NCT02608385	Advanced	SBRT	Pembrolizumab (anti-PD-1)
NCT03383302	Early stage	SBRT	Nivolumab (anti-PD-1)
NCT02400814	Advanced	SBRT	MPDL3280A (anti-PD-L1)

MATERIALS AND METHODS

Cells

Lewis lung carcinoma (LLC) cell line was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The LLC cells were cultured in DMEM medium supplemented with fetal bovine serum (10%), streptomycin (100 μl/ml) and penicillin (100 units/ml) (all from HyClone™ Laboratories, Logan, UT).

Animals

Female C57BL/6 mice, 6–8 weeks of age, were purchased from Center for Disease Control and Prevention of Hubei Province (Wuhan, China) and housed in a specific pathogen-free, temperature and humidity-controlled environment with food and water in their cages. All animal experiments were performed according to Wuhan University Animal Care Facility and National Institutes of Health guidelines.

The GFP-labeled LLC cells were orthotopically injected into the left lungs of C57BL/6 mice. The growth of the orthotopic tumors was analyzed using an *in vivo* fluorescence imaging system for small animals (Bruker Xtreme™ BI; Billerica, MA). Seven days after injection, anti-programmed death-1 (anti-PD-1) antibodies (200 μg/mouse, clone RMP1-14; Bio X Cell®, West Lebanon, NH) were injected intraperitoneally on days 7, 10, 13 and 16. Mice were anaesthetized and received 8 Gy thoracic irradiation on days 7, 10 and 13 using a small animal micro-CT irradiator (X-RAD 225Cx; Precision X-ray Inc., North Branford, CT). The beam was 225-kV photon at a dose rate of 3.12 Gy/min. The source-surface distance was 30 cm. The radiation field was 2×2 cm. Mice were sacrificed by cervical dislocation and the lungs were removed for further studies 18 days after injection (Fig. 1A). Each group was comprised of at least 6 mice for the murine experiment, and the entire experiment was repeated three times. To obtain the survival curve, 24 C57BL/6 female mice were divided into 4 groups: no treatment; anti-PD-1; irradiation; and combined irradiation and anti-PD-1. The data were collected and the survival curve was drawn using GraphPad Prism version 7.0 (LaJolla, CA).

Histopathology and Inflammation Grades

The fixed left lung tissue was embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining. Scoring was performed by two independent investigators in a blinded manner. To evaluate the degree of inflammation, the following criteria were used: 0 = no lung abnormality; 1 = presence of inflammation involving <25% of the lung parenchyma; 2 = lesions involving 25–50% of the lung; 3 = lesions involving >50% of the lung (*20*).

Immunohistochemical Analysis

Paraffin sections of lung tissue were deparaffinized. Goat serum (Boster Biological Technology, Pleasanton, CA) was used to block nonspecific binding sites. The sections were incubated with several primary antibodies, including myeloperoxidase (MPO; 1:4000) and CD4 (1:800) (both from Abcam®, Cambridge, MA) and CD8 (1:400; Cell Signaling Technology® Inc., Danvers, MA). The staining of infiltrated cells was examined in at least five random and representative fields from each section.

Cytometric Bead Array

Cytometric bead array was used to detect the serum levels of cytokines in the mice (Supplementary material Result of CBA: http://dx.doi.org/10.1667/RR15182.1.S1). The captured beads, containing interferon-γ (IFN-γ), tumor necrosis factor (TNF) and interleukin-5 (IL-5), were blended thoroughly. The mixed microsphere (50 μl) was incubated with 50 μl serum of cytokines and 50 μl PE-labeled test reagent for 3 h at room temperature. The beads were analyzed on FACScan™ using BD™ Cytometric Bead Array analysis software (Franklin Lakes, NJ).

Statistical Analysis

Student's *t* test was used for the comparisons of histopathological grade and serum cytokines (Supplementary material Ratio of CD4 CD8: http://dx.doi.org/10.1667/RR15182.1.S1). The log-rank test was used to evaluate survival time for different groups. Kruskal-Wallis H test was used for the MPO-positive rate and CD4/CD8 ratio. Significance was considered at $P < 0.05$. The analysis was performed

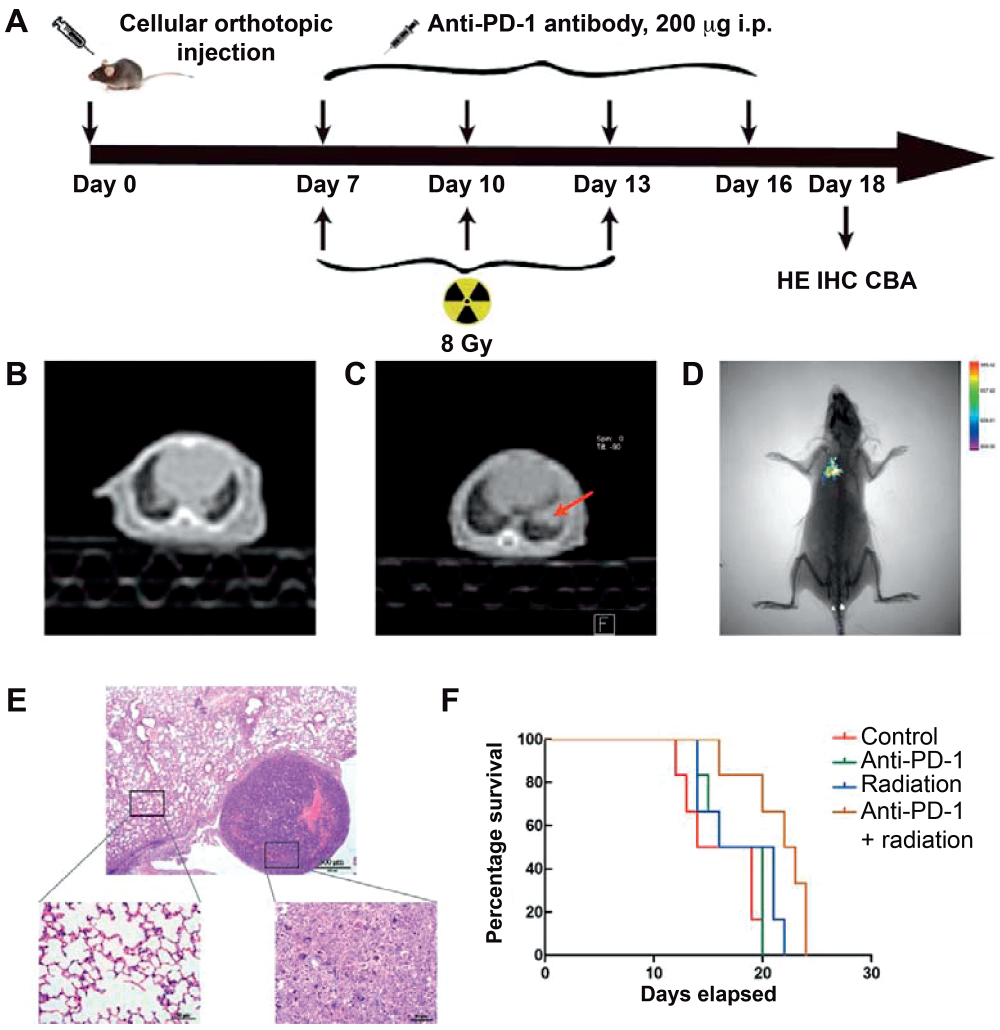

FIG. 1. Establishment of orthotopic lung cancer model of mice. Panel A: After the initial orthotopic tumor cell injection, mice were intraperitoneally injected with anti-PD-1 antibodies or vehicle control on days 7, 10, 13 and 16 (n = 6). Mice were anaesthetized and received received 8 Gy thoracic irradiation on days 7 10, 13 (n = 6). Panel B: Representative thin-layer scanning CT image of the lung of a non-tumor-bearing mouse. Panel C: Representative thin-layer scanning CT image of the lung of a tumor-bearing mouse. The left lung of the mouse clearly shows infiltration (red arrow). Panel D: Representative fluorescent image of a tumor-bearing mouse. Panel E: H&E stained tumor and normal lung tissues (upper, ~100Å; lower, ~400Å). Panel F: Survival curves of different treatment groups (n = 6). The combined treatment group had significantly longer survival ($P = 0.015$ vs. control; $P = 0.049$ vs. radiation; $P = 0.029$ vs. anti-PD-1).

using SPSS® version 11.0 (Chicago, IL) and the graph was created using GraphPad version 7.0.

RESULT

Establishment of the Orthotopic Lung Cancer Mouse Model

Seven days after tumor cell injection, mice were anesthetized for CT scanning to determine whether the orthotopic lung cancer model was established. As expected, chest scan showed that compared to the image of the lung of a normal mouse (Fig. 1B), the left lung of the tumor-injected mouse clearly showed infiltration (Fig. 1C). The location of the fluorescent signal was consistent with the site of the previous operation (Fig. 1D). No swollen lymph nodes were found in the thoracic cavity, and there was nodular tissue on the surface of the left lung. H&E staining showed the tumor tissue was just on the surface of the lung tissue. The membrane of tumor tissue was intact, in which there was bleeding and hemorrhagic necrosis. The neoplastic cells showed atypia, with hyperchromatic nucleus, which was clearly different from the adjacent lung tissue (Fig. 1E).

Effects of Anti-PD-1 Treatment Combined with Radiotherapy on Lung Inflammation

As demonstrated by the survival curve, the effect of anti-PD-1 combined with radiation significantly improved the survival rate of mice with orthotopic lung cancer. Compared to the control group, the overall survival from combined treatment was longer and the difference was statistically

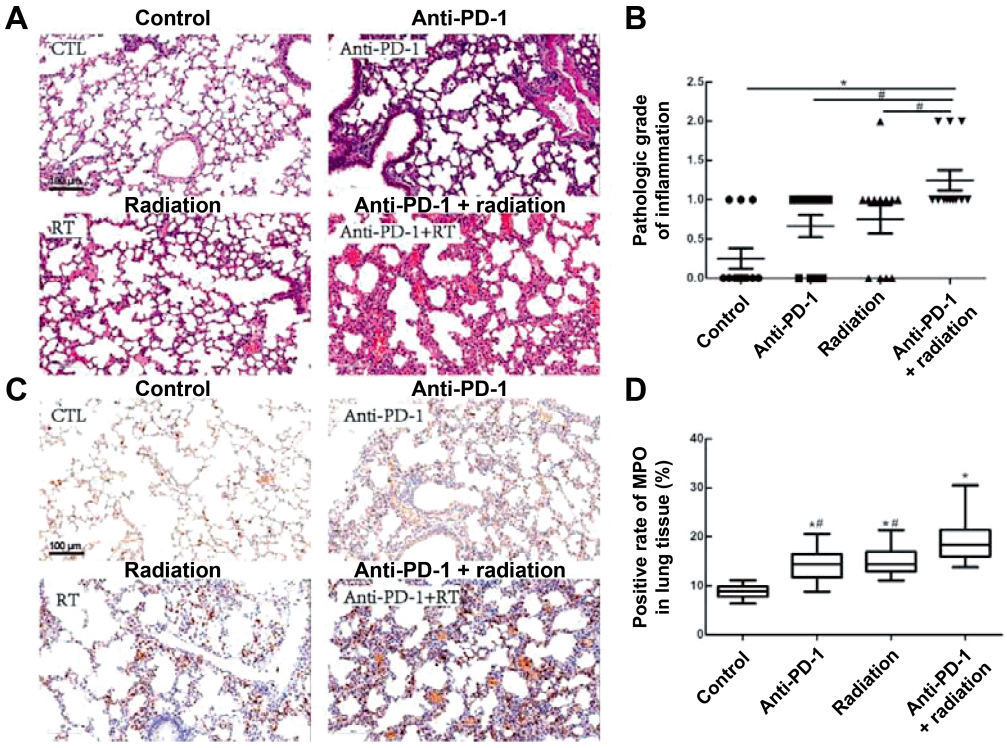

FIG. 2. Effects of anti-PD-1 treatment combined with radiation on lung inflammation. Panel A: Representative images of H&E stained normal lung tissue from mice with different treatments. Panel B: The pathologic grade of inflammation was quantified. The combined treatment group showed the highest score of pneumonia. Panel C: Representative images of IHC staining for MPO in normal lung tissues from mice with different treatments. Panel D: Percentages of MPO-positive cells were quantified. The combined treatment group showed the highest percentage. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. anti-PD-1 combined with radiation.

significant. Moreover, the overall survival in mice receiving combined treatment was also longer than those in the groups receiving either single anti-PD-1 or irradiation (Fig. 1F). We considered the possibility that the mice might die due to cachexia or respiratory failure. These results suggested that the combined treatment was effective. The histological score of H&E staining (Fig. 2A) showed the highest score of pneumonitis in the combined treatment group (Fig. 2B). We next used immunohistochemical to confirm the pneumonitis evaluation with an antibody against MPO (*21*) (Fig. 2C). As expected, the percentage of MPO-positive cells in the lung of mice receiving combined treatment was the highest among the four groups (Fig. 2D).

The CD4/CD8 T-Cell Ratio Increased in Lung Tissue but Decreased in Orthotopic Tumor after Combined Treatment

Immunohistochemical staining for CD4 and CD8 was performed in the lung (Fig. 3A and B) and tumor tissues (Fig. 3C and D) of the four treatment groups. After counting the number of CD4 T cells and CD8 T cells in the same areas of immunohistochemical staining for anti-CD4 and anti-CD8, we found that the CD4/CD8 ratio was significantly decreased in the combined treatment group (Fig. 3E). When the CD4/CD8 ratio in lung tissues of the four groups was examined, it was found to be moderately increased in the three experimental groups; however, only the increase in the ratio of the combined treatment group was statistically significantly different from the control group (Fig. 3F). These results indicate that the combined treatment had different effects on tumor and adjacent lung tissues.

Serum Cytokine Levels Increased after Combined Treatment

The serum levels of IFN-γ were dramatically increased upon combined treatment with anti-PD-1 and radiation. There was significant difference between the combined treatment group and the other three groups. The levels of IFN-γ were also moderately increased in groups treated with anti PD-1 or radiation compared to the control group, but the increase was not statistically significant (Fig. 3G). Significant increase in TNF and IL-5 levels was also observed in the combined treatment group when compared to the other three groups (Fig. 3H and I).

DISCUSSION

This study revealed the more severe influence on lung tissues when concurrent treatment with anti-PD-1 and radiation is applied. The PD-1 therapy regimen was chosen for this work based on previously published studies showing the efficacy of PD-1 in the treatment of subcutaneous tumor (*22*). For irradiations, the regimen of

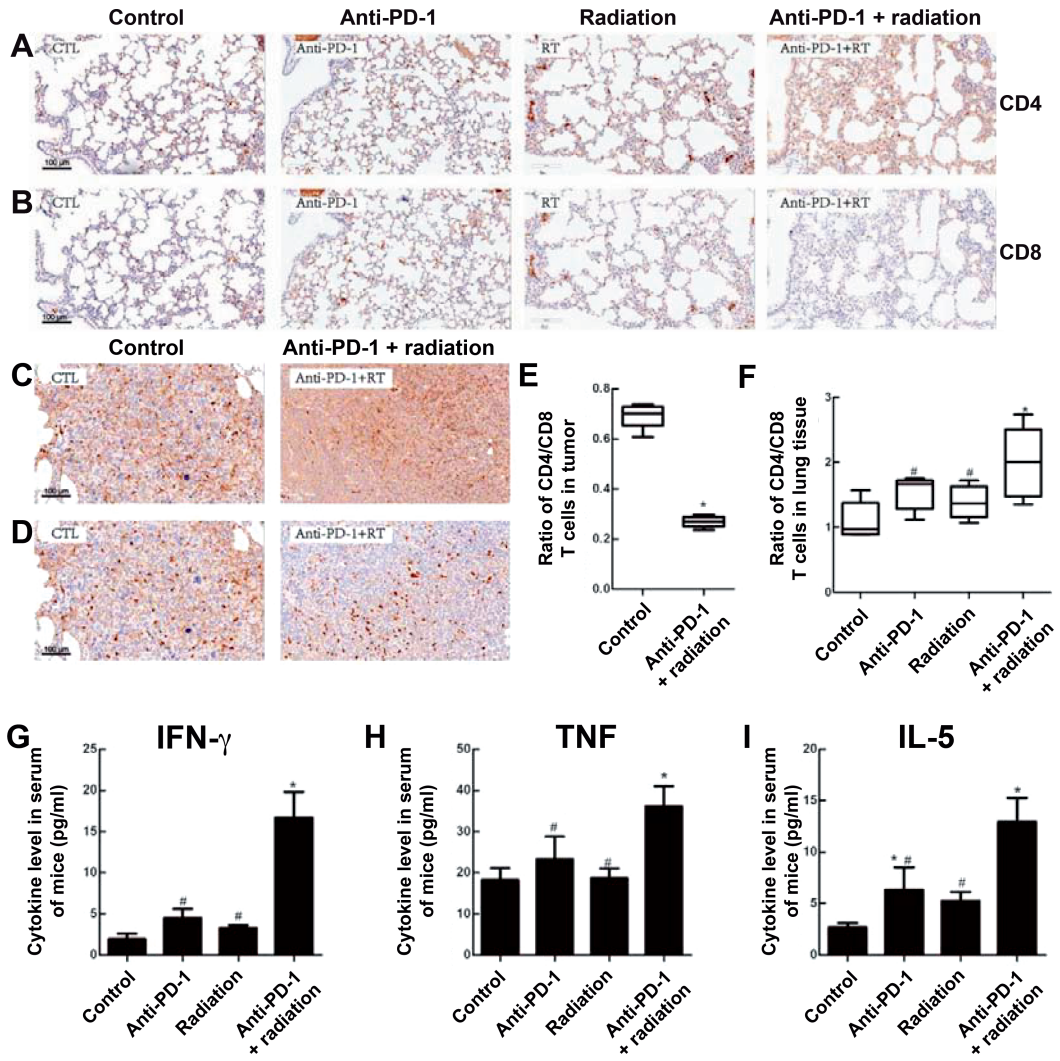

FIG. 3. Changes in CD4/CD8 ratio and cytokines of mice receiving combined treatment. Panel A: Representative images of IHC staining of CD4 in normal lung tissues from mice with different treatments. Panel B: Representative images of IHC staining of CD8 in the normal lung tissues from mice with different treatments. Panel C: Representative images of IHC staining of CD4 in the tumor tissues from mice with combined therapy or no treatment. Panel D: Representative images of IHC staining of CD8 in the tumor tissues from mice with combined therapy or no treatment. Panel E: The CD4/CD8 ratios in the tumor tissues were significantly lower in the combined therapy group than those in the control group. Panel F: CD4/CD8 ratios in the normal lung tissues from mice with different treatments. The ratios were significantly higher in the combined group. Panel G: The serum levels of IFN-γ were significantly higher in the combined treatment group. Panel H: Serum levels of TNF were significantly higher in the combined treatment group. Panel I: Serum levels of IL-5 were significantly higher in the combined treatment group. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. anti-PD-1 with radiation.

8 Gy × 3 doses was selected based on the published literature suggesting, for that regimen, the body had the strongest immunogenicity (*23*). We expected that 8 Gy radiation × 3 doses induced optimal function of the immune checkpoint inhibitor.

The results of the current work showed that the group receiving combined treatment had the highest lung injury score compared to the control or single treatment groups, indicating that the combined treatment caused more severe damage to the lung tissue than the single treatment with either radiation or anti-PD-1. Myeloperoxidase is released by the neutrophils and considered to be a specific marker of myeloid cells (*24*). It was reported as an important marker for the degree of inflammatory response (*25*). The infiltration of neutrophils reflects the severity of lung injury to a certain extent. The changing trend of the positive rate of MPO was the same as that of the grade of lung injury, suggesting that the experimental results of lung injury were validated effectively.

The CD4/CD8 ratio is one of the indicators of change in T-cell subsets. A ratio imbalance is one of the pathogenic factors of many diseases, especially autoimmune diseases. In studies published elsewhere, this ratio was shown to be significantly higher in the blood of patients with rheumatoid

arthritis, ulcerative colitis and polymyositis and multiple sclerosis (*26*) compared to healthy individuals. These results suggested that the increase in the ratio resulted in a disorder of the immune system and caused damage to different target organs. Published studies of cutaneous toxicity caused by many immune checkpoint inhibitors have suggested that the treatments led to mixed CD4/CD8 ratio or a predominantly $CD4^+$ T-cell infiltration (*27–30*). However, the mechanism of pneumonitis caused by immune checkpoint inhibitor is not clear. Previous studies have also indicated that $CD4^+$ T cells play a leading role in the development of radiation pneumonitis (*31*). The process in the imbalance involved two subsets of $CD4^+$ T cells: Th1 and Th2 cells (*32*). In this experiment, the ratios in the single-treatment groups (radiation or anti-PD-1) were not significantly different from that in the control group. However, the increased ratio in lung tissue of the combined treatment group might be associated with increased $CD4^+$ T-cell infiltration in the lung tissue, resulting in a higher rate of inflammation and eventually inducing the lung tissue injury.

The CD4/CD8 ratio in the tumor tissue of the combined treatment group is significantly reduced compared to that in the control group, which might be related to $CD8^+$ T-cell activation in the tumor tissue. Some published studies have suggested that a better prognosis of gallbladder carcinoma was related to the high $CD8^+$ T-cell infiltration and low $CD4^+$ T-cell infiltration in the tumor tissue (*33*). The clinical trials of targeted PD-1 therapy confirmed that the killing effect of the tumor was mediated by $CD8^+$ T cells (*34*). The depletion of $CD4^+$ T cells during targeting PD-1 therapy increased $CD8^+$ T-cell infiltration in the tumor tissue (*35*). Therefore, the decrease of the ratio in the tumor tissue caused by combined treatment might be related to the anti-tumor effect mediated by $CD8^+$T cells.

Changes in cytokines, including IFN-γ, TNF and IL-5, were partly consistent with changes in the grades of lung injury and the CD4/CD8 ratio between the control group and the combined treatment group. While the changes in each of the four groups were different, the trend indicated that the response mechanism of the combined therapy promoted the release of inflammatory factors and increased the risk of damage to the normal organs. In another published study, radiation-induced heart damage was shown to be regulated by the PD-1 pathway. Anti-PD-1 therapy and chest radiotherapy aggravated heart damage. The findings of that study are considered as the basis for reduced radiotherapy doses in the setting of combined therapy (*36*). In addition, a retrospective clinical study showed that in patients receiving a single drug, Optivo, the overall response rate was significantly higher in patients with immune-related adverse events than in those without them (52.3% vs. 27.9%), and the progress free survival was also twice as much as the latter (9.2 months vs. 4.8 months) (*37*). These results suggested that the combination of radiotherapy and immune checkpoint inhibitors made the tumor more sensitive to the immune system while the normal tissue was under a higher risk of being attacked.

The PACIFIC study by Antonia *et al*. (*38*) demonstrated no difference in severe (grade 3) pneumonitis rates in the control arm compared to chemoradiotherapy combined with adjuvant anti-PD-L1 therapy, which is not supportive of our results. However, the patients in PACIFIC clinical trial received concurrent chemotherapy and radiotherapy before they received the anti-PD-L1 treatment, while the treatment of our study was the simultaneous application of radiotherapy and immune checkpoint inhibitors. Moreover, the PACIFIC study used PD-L1 inhibitors, whereas our experiment used PD-1 inhibitors; the results showed that in the PACIFIC study, lung injury caused by the use of PD-L1 inhibitors with radiotherapy was not obvious, while in the current study, PD-1 inhibitors combined with radiation led to significant lung injury.

In this study, we explored the effect of radiation combined with anti-PD-1 antibodies using an orthotropic tumor-bearing mouse model. There are some limitations to this work, however. Although a series of phenotypes were detected, the intrinsic mechanism requires further exploration. Only one radiation type, dose and timing was employed in this work. To confirm our findings, evaluations using other radiation scenarios are needed. Furthermore, these studies used an animal model, which can only be validated at the animal level. Further verification is required for potential applications in a clinical setting. Since these results indicate that the combined treatment with anti-PD-1 antibodies and radiation induced significantly higher serum levels of cytokines, including IFN-γ, TNF and IL-5, we expect similar results from currently ongoing human clinical trials; we surmise that the higher levels of these cytokines also suggest increased severity of lung injury induced by both irradiation and anti-PD-1 antibodies. This work provides a novel perspective from which physicians may consider immunoradiotherapy for tumor patients.

ACKNOWLEDGMENTS

This study was supported in part by grants from the National Nature Science Foundation of China (grant nos. 81572967, 81372498, 81800429 and 81773236), Hubei Natural Science Foundation (grant no. 2013CFA006), Zhongna Hospital of Wuhan University Science, Technology and Innovation Seed Fund (grant nos. znpy2016050, 2017001, 2017049, 2018028, and 2018070), National Key Clinical Specialty Construction Program of China [no. (2013)544], Wuhan City Huanghe Talents Plan. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Received: July 13, 2018; accepted: January 10, 2019; published online: January 29, 2019

REFERENCES

1. Tandberg DJ, Tong BC, Ackerson BG, Kelsey CR. Surgery versus stereotactic body radiation therapy for stage I non-small cell lung cancer: A comprehensive review. Cancer 2018; 124:667–78.

2. Kalemkerian GP. Small cell lung cancer. Semin Respir Crit Care Med 2016; 37:783–96.
3. Sangro B, Martinez-Urbistondo D, Bester L, Bilbao JI, Coldwell DM, Flamen P, et al. Prevention and treatment of complications of selective internal radiation therapy: Expert guidance and systematic review. Hepatology 2017; 66:969–82.
4. Hinojosa CA, Soni NJ, Anzueto A, Babu BL, Gonzalez-Juarbe N, Rodriguez AH, et al. Prevention and treatment of complications of selective internal radiation therapy: Expert guidance and systematic review. Am J Respir Crit Care Med 2017; 66:969–82.
5. Marks LB, Yu X, Vujaskovic Z, Small W Jr., Folz R, Anscher MS. Radiation-induced lung injury. Semin Radiat Oncol 2003; 13:333–45.
6. Mehta V, Radiation pneumonitis and pulmonary fibrosis in non-small cell lung cancer: pulmonary function, prediction, and prevention. Int J Radiat Oncol Biol Phys 2005; 63:5–24.
7. Simone CB 2nd. Thoracic radiation normal tissue injury. Semin Radiat Oncol 2017; 27:370–7.
8. Chen J, Tian X, Mei Z, Wang Y, Yao Y, Zhang S, et al. The effect of the TLR9 ligand CpG-oligodeoxynucleotide on the protective immune response to radiation-induced lung fibrosis in mice. Mol Immunol 2016; 80:33–40.
9. Callahan MK, Postow MA, Wolchok JD. Targeting T cell co-receptors for cancer therapy. Immunity 2016; 44:1069–78.
10. Shin SJ, Jeon YK, Kim PJ, Cho YM, Koh J, Chung DH, et al. Clinicopathologic analysis of PD-L1 and PD-L2 expression in renal cell carcinoma: association with oncogenic proteins status. Ann Surg Oncol 2016; 23:694–702.
11. Wang W, Wang K, Chen Z, Chen L, Guo W, Liao P, et al. Immunoclassification characterized by CD8 and PD-L1 expression is associated with the clinical outcome of gastric cancer patients. Oncotarget 2018; 9:12164–73.
12. Furue M, Ito T, Wada N, Wada M, Kadono T, Uchi H. Melanoma and immune checkpoint inhibitors. Curr Oncol Rep 2018; 20:29.
13. Horn L, Spigel DR, Vokes EE, Holgado E, Ready N, Steins M, et al. Nivolumab versus docetaxel in previously treated patients with advanced non-small cell lung cancer: Two-year outcomes from two randomized, open-label, phase iii trials (CheckMate 017 and CheckMate 057). J Clin Oncol 2017; 35:3924–33.
14. Escudier B, Sharma P, McDermott DF, George S, Hammers HJ, Srinivas S, et al. CheckMate 025 randomized phase 3 study: Outcomes by key baseline factors and prior therapy for nivolumab versus everolimus in advanced renal cell carcinoma. Eur Urol 2017; 72:962–71.
15. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small cell lung cancer. N Engl J Med 2015; 373:1627–39.
16. Nishino M, Giobbie-Hurder A, Hatabu H, Ramaiya NH, Hodi FS. Incidence of programmed cell death 1 inhibitor-related pneumonitis in patients with advanced cancer: A systematic review and meta-analysis. J JAMA Oncol 2016; 2:1607–16.
17. Zhao J, Yorke ED, Li L, Kavanagh BD, Li XA, Das S, et al. Simple factors associated with radiation-induced lung toxicity after stereotactic body radiation therapy of the thorax: A pooled analysis of 88 studies. Int J Radiat Oncol Biol Phys 2016; 95:1357–66.
18. Shepherd FA, Douillard JY, Blumenschein GR. [Immunotherapy for non-small cell lung cancer–novel approaches to improve patient outcome]. Zhongguo fei ai za zhi 2013; 16:C9–20.
19. Shaverdian N, Lisberg AE, Bornazyan K, Veruttipong D, Goldman JW, Formenti SC, et al. Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial. Lancet Oncol 2017; 18:895–903.
20. Harada C, Kawaguchi T, Ogata-Suetsugu S, Yamada M, Hamada N, Maeyama T, et al. EGFR tyrosine kinase inhibition worsens acute lung injury in mice with repairing airway epithelium. Am J Respir Crit Care Med 2011; 183:743–51.
21. Chen Z, Shao X, Dou X, Zhang X, Wang Y, Zhu C, et al. Role of the mycoplasma pneumoniae/interleukin-8/neutrophil axis in the pathogenesis of pneumonia. PloS One 2016; 11:e0146377.
22. Shi L, Chen L, Wu C, Zhu Y, Xu B, Zheng X, et al. PD-1 blockade boosts radiofrequency ablation-elicited adaptive immune responses against tumor. Clin Cancer Res 2016; 22:1173–84.
23. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun 2017; 8:15618.
24. Moradi M, Mojtahedzadeh M, Mandegari A, Soltan-Sharifi MS, Najafi A, Khajavi MR, et al. The role of glutathione-S-transferase polymorphisms on clinical outcome of ALI/ARDS patient treated with N-acetylcysteine. Respir Med 2009; 103:434–41.
25. Tanaka S, Nishiumi S, Nishida M, Mizushina Y, Kobayashi K, Masuda A, et al. Vitamin K3 attenuates lipopolysaccharide-induced acute lung injury through inhibition of nuclear factor-kappaB activation. Clin Exp Immunol 2010; 160:283–92.
26. Carotenuto A, Scalia G, Ausiello F, Moccia M, Russo CV, Sacca F, et al. CD4/CD8 ratio during natalizumab treatment in multiple sclerosis patients. J Neuroimmunol 2017; 309:47–50.
27. Lacouture ME, Wolchok JD, Yosipovitch G, Kahler KC, Busam KJ, Hauschild A. Ipilimumab in patients with cancer and the management of dermatologic adverse events. J Am Acad Dermatol 2014; 71:161–9.
28. Shi VJ, Rodic N, Gettinger S, Leventhal JS, Neckman JP, Girardi M, et al. Clinical and histologic features of lichenoid mucocutaneous eruptions due to anti-programmed cell death 1 and anti-programmed cell death ligand 1 immunotherapy. JAMA Dermatol 2016; 152:1128–36.
29. Joseph RW, Cappel M, Goedjen B, Gordon M, Kirsch B, Gilstrap C, et al. Lichenoid dermatitis in three patients with metastatic melanoma treated with anti-PD-1 therapy. Cancer Immunol Res 2015; 3:18–22.
30. Schaberg KB, Novoa RA, Wakelee HA, Kim J, Cheung C, Srinivas S, et al. Immunohistochemical analysis of lichenoid reactions in patients treated with anti-PD-L1 and anti-PD-1 therapy. J Cutan Pathol 2016; 43:339–46.
31. Toma CL, Serbescu A, Alexe M, Cervis L, Ionita D, Bogdan MA. The bronchoalveolar lavage pattern in radiation pneumonitis secondary to radiotherapy for breast cancer. Maedica (Buchar) 2010; 5:250–7.
32. Chen J, Wang Y, Mei Z, Zhang S, Yang J, Li X, et al. Radiation-induced lung fibrosis in a tumor-bearing mouse model is associated with enhanced type-2 immunity. J Radiat Res 2016; 57:133–41.
33. Fluxa P, Rojas-Sepulveda D, Gleisner MA, Tittarelli A, Villegas P, Tapia L, et al. High CD8(+) and absence of Foxp3(+) T lymphocytes infiltration in gallbladder tumors correlate with prolonged patients survival. BMC Cancer 2018; 18:243.
34. Buchan SL, Fallatah M, Thirdborough SM, Cabiddu M, Penfold CA, He LZ, et al. PD-1 blockade and CD27 stimulation activate distinct transcriptional programs that synergize for CD8(+) T-cell-driven antitumor immunity. Clin Cancer Res 2018; 24:2383–94.
35. Ueha S, Yokochi S, Ishiwata Y, Ogiwara H, Chand K, Nakajima T, et al. Robust antitumor effects of combined anti-CD4-depleting antibody and anti-PD-1/PD-L1 immune checkpoint antibody treatment in mice. Cancer Immunol Res 2015; 3:631–40.
36. Du S, Zhou L, Alexander GS, Park K, Yang L, Wang N, et al. PD-1 modulates radiation-induced cardiac toxicity through cytotoxic T lymphocytes. J Thorac Oncol 2018; 13:510–20.
37. Haratani K, Hayashi H, Chiba Y, Kudo K, Yonesaka K, Kato R, et al. Association of immune-related adverse events with nivolumab efficacy in non-small cell lung cancer. JAMA Oncol 2018; 4:374–8.
38. Uemura T, Hida T. Durvalumab showed long and durable effects after chemoradiotherapy in stage III non-small cell lung cancer: results of the PACIFIC study. J Thorac Dis 2018; 10:S1108–12.