

Suppression of Atopic Dermatitis and Tumor Metastasis in Mice by Small Amounts of Radon

Mareyuki Takahashi¹ and Shuji Kojima

Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

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We examined the effect of radon in two experimental disease models in mice by administering radon dissolved in water at 68–203 Bq/liter. Administration of radon in drinking water to NC/Nga mice significantly delayed the progression of atopic dermatitis-like skin lesions induced by picrylchloride when administered prior to the induction of disease signs. The number of pulmonary metastatic foci in C57BL/6 mice inoculated with B16 melanoma cells was also reduced significantly by administration of radon in drinking water when the number of tumor cells was small and the radon treatment was started prior to tumor inoculation. The ratio of Ifng to Il4 produced by splenocytes from BALB/c mice immunized with DNP-Ascaris was significantly increased by administration of radon in drinking water. From these results, a modulation of immunity by radon was suggested. © 2006 by Radiation Research Society

INTRODUCTION

Radon (^{222}Rn , $t_{1/2}$ 3.82 = days, α -particle energy = 5.49 MeV) is a gaseous radioactive element that is ubiquitously present in the atmosphere, contributing to about half the background radiation. At high concentrations, radon is particularly damaging (1–3) and is considered as an environmental hazard (4, 5), whereas at low concentrations it has been used as a folk remedy. Although some clinical reports on the therapeutic use of radon inhalation are available (6, 7), the underlying mechanism remains to be elucidated.

Radon-222 and its short-lived decay products, ^{218}Po ($t_{1/2}$ = 3.11 min, 6.00 MeV) and ^{214}Po ($t_{1/2}$ = 164 μs , 7.69 MeV), are α -particle-emitting radionuclides that are responsible for the biological effects of radon. A characteristic feature of α -particle radiation in tissues is that only cells located within 30–80 μm of the α -particle emitters are traversed. An α particle from radon deposits an absorbed energy of 0.1–0.5 Gy in the traversed cells, creating a microscopic irradiated field in the tissues. Although in-

halation has been regarded as the major route for introducing radon into the body, radon dissolved in drinking water is also effectively absorbed from the stomach wall and enters the blood circulation (8). Most ingested radon circulates in the blood for about 1 h before it is exhaled from the lungs (9). Using water containing radon, we examined the effects of radon in two disease models in which modulation of immune cell function has a significant therapeutic impact.

Atopic dermatitis-like skin lesions in NC/Nga mice are an animal model for human atopic dermatitis (10). The mice have a strong tendency of shifting to a Th2-dominant state after contact with some environmental factors or artificial antigens (11). The immunopathological features of the mice are characterized by defective production of Ifng by T cells due to low sensitivity to Il12 as well as decreased responsiveness of B cells to Ifng (12), which are supposed to be responsible for hyperproduction of IgE.

The metastasis of B16 melanoma in syngeneic C57BL/6 mice represents an important model system for T-cell-based tumor immunity. In this model, Th2-dominant immunity is established upon inoculation of B16 melanoma cells, and the severity of metastasis is strongly influenced by Il4 released from tumor-associated Th2 cells (13).

We report here that ingested radon significantly ameliorated the disease signs in these animal models when it was administered before the immunity pattern had been fully established. Modulation of the immune balance by radon was considered.

MATERIALS AND METHODS

Reagents and Cells

Picrylchloride (2,4,6-trinitrochlorobenzene) was purchased from Tokyo Kasei (Tokyo, Japan) and DNP-Ascaris from Cosmobio (Tokyo, Japan). The antibodies used in ELISA, affinity purified and biotinylated rat anti-mouse Il4 and affinity purified and biotinylated rat anti-mouse Ifng, were purchased from eBioscience (San Diego, CA). Recombinant Ifng and recombinant Il4 were from eBioscience (San Diego, CA). B16 melanoma cells were purchased from Health Science Research Resources Bank (Osaka, Japan).

Administration of Water Containing Radon

As a source of ingested radon, natural water officially approved for drinking in the northern part of the Kyusyu area of Japan was used. The

¹ Address for correspondence: Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan; e-mail: mtaka@rs.noda.tus.ac.jp.

concentration of radon in the water was 430 Bq/liter at the water source. The water was filter-sterilized and freshly supplied once a week. The radon concentration in the water was 203 Bq/liter at the beginning of the week and 68 Bq/liter at the end of the week. Radon-half-depleted water [Rn(1/2)] and radon-depleted water [Rn(-)] were prepared by allowing the water to stand for 4 and 30 days, respectively, before use. The approximate amount of radon ingested by each mouse was 140–176, 68–85 and 0.86–1.08 Bq/kg week⁻¹ for radon, Rn(1/2) and Rn(-), respectively.

Mice were treated and handled according to the Guide Principles for the Care and Use of Laboratory Animals of the Japanese Pharmacological Society. All experiments were approved by Institutional Animal Care and Use Committee of Tokyo University of Science.

Atopic Dermatitis

Five-week-old female SPF NC/Nga mice (Charles River Japan, Kanagawa) were used. For sensitization of mice (14), purified picrylchloride dissolved in the solvent (ethyl alcohol:acetone = 4:1) at a concentration of 5% (w/v) was applied to each mouse's hair-trimmed chest, abdomen and footpad. Four days later the mice were challenged with picrylchloride dissolved in olive oil at a concentration of 1% (w/v) onto the both ears and the back. The challenge treatment was repeated once a week. Control mice received the same treatments without picrylchloride. Five signs of dermatitis and edema, excoriation/erosion, erythema/hemorrhage, dryness/scaling and scratching, were graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). The sum of the individual scores was expressed as a total clinical severity score (15).

For measurement of IgE, blood samples were collected from the retro-orbital plexus, and plasma samples were stored at -80°C until analysis. The assay was performed using an IgE ELISA Quantitation kit (Bethyl Laboratories, Montgomery, TX).

Lung Metastasis

Six-week-old male C57BL/6 mice (Japan SLC, Hamamatsu) were supplied with water containing radon, Rn(1/2), Rn(-) or distilled water starting 2 weeks prior to the injection of tumor cells and continuing throughout the experiments. B16 melanoma cells were cultured in RPMI 1640 medium supplemented with 5% FBS. B16 cells suspended in 0.2 ml PBS at appropriate concentrations were injected into the tail veins of the mice. Fourteen days after B16 inoculation, lung tissues were removed and visible metastatic colonies were counted. In the delayed-administration experiments, the administration of drink water containing radon was started immediately after B16 inoculation and continued throughout the experiment.

Immunization of Mice with DNP-Ascaris

Eight-week-old male BALB/c mice (Japan SLC) were supplied with either Rn(-), Rn(1/2) or water containing radon starting 2 weeks prior to the immunization and continuing throughout the experiments. Mice were immunized by i.p. injection of 10 µg DNP-Ascaris adsorbed with 4 mg of alum on day 0. Control mice were injected with saline. On day 10, blood samples were taken for IgE analysis and splenocytes were then isolated and cultured in RPMI 1640 medium with 10% FBS at 5 × 10⁶ cells/ml in the presence of 10 µg/ml of DNP-Ascaris for 18 h. Cytokine levels in the culture supernatant were analyzed by sandwich ELISA. Plasma IgE was measured as described above.

Statistical Analysis

Statistical significance was determined using Student's *t* test or Dunnett's test for multiple comparisons. Differences in the severity scores in NC/Nga mice were analyzed by the Mann-Whitney *U* test. Differences were considered as significant at *P* < 0.05.

RESULTS

Atopic Dermatitis

We examined the effect of ingestion of radon in SPF NC/Nga mice with hapten-induced dermatitis. Ingestion of radon was started 2 weeks prior to the sensitization with picrylchloride and continued throughout the experiment. The first skin lesions appeared 2 weeks after the start of the challenge treatment and progressed thereafter (Fig. 1A). Four weeks after the first challenge, the difference between mice that received radon and the Rn(-) mice became statistically significant. The plasma IgE levels in the mice that ingested radon measured 4 weeks after the first challenge were significantly lower than those in Rn(-) mice (Fig. 1B).

To determine the most effective time for administration of radon, we also administered radon after the challenge treatment had already started. This was done in the same experiment shown in Fig. 1A. One week after the first challenge, radon ingestion was started (indicated by an arrow); no effect of radon on disease progression was observed (Fig. 1C). Plasma IgE levels were not different in mice given radon and Rn(-) mice (Fig. 1D).

Tumor Metastasis

The effect of radon on the cell-mediated immunity against the syngeneic B16 melanoma cells in C57BL/6 mice was examined. Radon treatment was started 2 weeks prior to the injection of different numbers of tumor cells. Two weeks after injection, metastatic colonies in lungs were counted. When 2 × 10⁵ cells [Fig. 2A (1)] or 1 × 10⁵ cells [Fig. 2A (2)] were injected, similar numbers of lung colonies formed in mice given radon and in Rn(-) mice; significantly fewer colonies were observed in mice given radon than in Rn(-) mice when 5 × 10⁴ cells were injected [Fig. 2A (3)]. In separate experiments, the effect of radon half-depleted water [Rn(1/2)], in which about half the amount of radon was present, were also evaluated using 5 × 10⁴ tumor cells. Water containing the full amount of radon effectively suppressed the lung metastasis, whereas water half-depleted of radon did not have a significant effect on metastasis (Fig. 2B), though the mean number of colonies decreased somewhat. The timing of radon treatment was also found to be important; the administration of radon right after tumor inoculation [Rn(d)] did not have a suppressive effect (Fig. 2C).

These results indicate that the anti-metastatic effect of ingested radon is observed when the number of tumor cells inoculated is small and, importantly, when the radon treatment is initiated prior to the tumor inoculation, suggesting that although the anti-metastatic effect may not be strong, it is significant when the tumor-dependent Th2 immunity is not yet fully established.

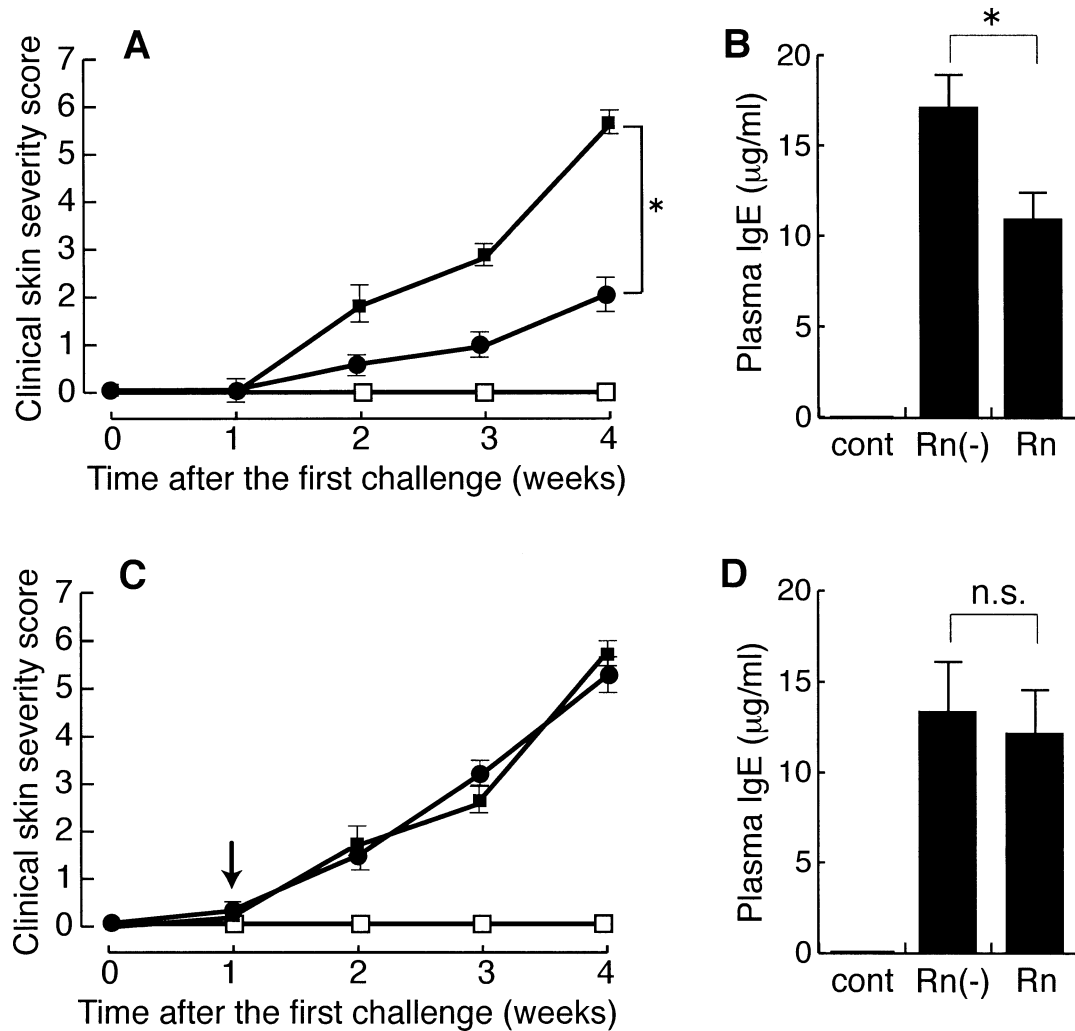


FIG. 1. Effect of radon on atopic dermatitis-like skin lesions induced by picrylchloride in SPF NC/Nga mice. Panel A: Progression of skin lesions. Radon treatment was started 2 weeks prior to sensitization with picrylchloride and continued throughout the experiment. Mice were challenged with picrylchloride once a week starting 4 days after sensitization. The severity of skin lesions was scored once a week. □: Control mice (cont) ($n = 5$); ■: Rn(-) mice ($n = 8$); ●: mice given radon (Rn) ($n = 9$). * $P < 0.05$. Data are representative of two separate experiments with similar results. Panel B: Plasma IgE was measured 4 weeks after the first challenge. * $P < 0.05$. Panel C: Effect of radon when treatment was delayed. Radon ingestion was started 1 week after the first challenge (as indicated with an arrow) and continued throughout the experiment. Other procedures are the same as described in panel A. □: Control mice ($n = 4$); ■: Rn(-) mice ($n = 6$); ●: mice given radon ($n = 6$). Panel D: Plasma IgE was measured 4 weeks after the first challenge in the experiment in panel C. n.s.: not significant. Each value represents the mean $\pm$ SEM.

Modulation of Th1/Th2 Balance by Radon in Mice Treated with DNP-*Ascaris*

We examined the effect of radon on the immunity balance in mice of the strong Th2-dominant strain, BALB/c. Mice were given either water containing radon, Rn(1/2) water or Rn(-) water for 2 weeks prior to the immunization with DNP-*Ascaris* as an antigen. Ten days after immunization, splenocytes were collected and cultured with antigen for 18 h. Splenocytes obtained from mice receiving radon produced significantly greater amounts of Ifng than those from Rn(-) mice (Fig. 3A), whereas Il4 levels were similar in both groups (Fig. 3B). The ratio Ifng/Il4 in-

creased significantly in mice given radon compared with Rn(-) mice (Fig. 3C), indicating that radon skewed the immunity balance toward Th1. Elevation of plasma IgE levels was also suppressed in mice given radon (Fig. 3D). Rn(1/2) did not have such effects.

DISCUSSION

Radon decays to emit an α particle with an energy of 5.49 MeV, which corresponds to an LET of 90 keV μm^{-1} . The track length of an α particle with this energy in biological tissue is about 50–60 μm , indicating that a single

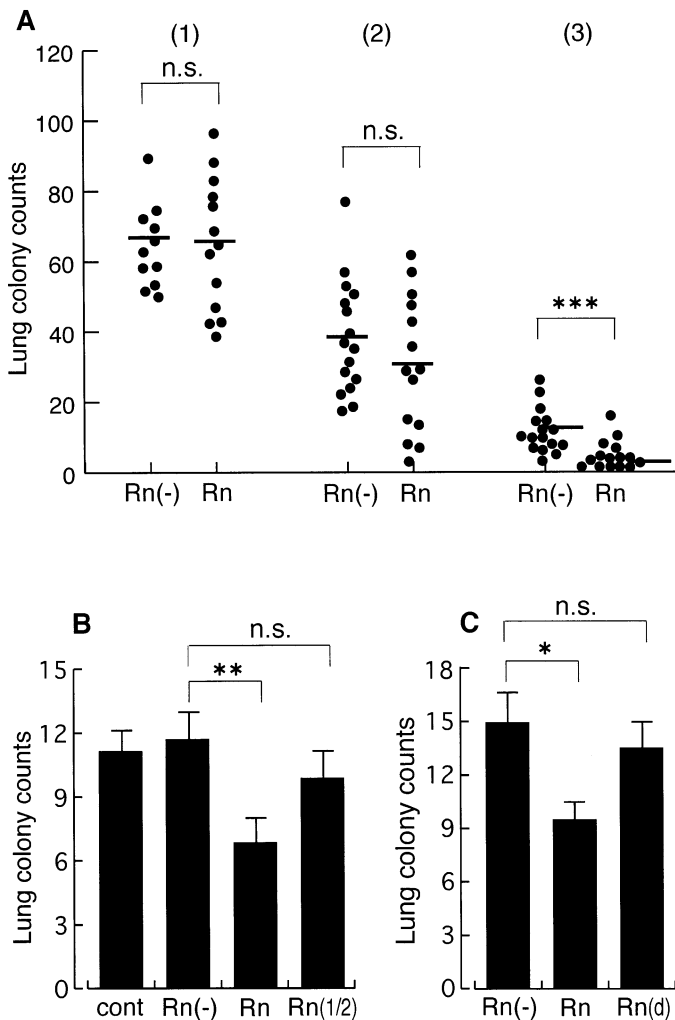


FIG. 2. Formation of metastatic lung colonies in C57BL/6 mice. Lung colonies were counted 2 weeks after injection of B16 melanoma cells. Panel A: Colony formation after injection of different numbers of B16 cells. Radon treatment was started 2 weeks prior to the injection of B16 cells and continued throughout the experiment. (1): 2×10^5 cells; Rn(-) ($n = 11$), mice given radon (Rn) ($n = 13$); (2): 1×10^5 cells; Rn(-) ($n = 16$), mice given radon ($n = 14$); (3): 5×10^4 cells; Rn(-) ($n = 16$), mice given radon ($n = 15$). The bars represent the means of the groups. n.s.: not significant. *** $P < 0.005$. Panel B: Effect of radon in the water administered to mice. 5×10^4 B16 cells were injected. Rn(1/2): radon-half-depleted water. n.s.: not significant. ** $P < 0.01$ ($n = 7$ each). Panel C: Effect of radon when treatment was delayed. Administration of radon to Rn(d) mice was started right after the injection of 5×10^4 B16 cells and continued throughout the experiment. n.s.: not significant. * $P < 0.05$ ($n = 6$ each). Each value represents the mean $\pm$ SEM.

α particle emitted from a radon molecule affects less than a few tens of cells located nearby. Since only a small number of cells are irradiated with high-energy α particles, the movement and localization of radon and its decay products in tissues are important. A portion of ingested radon is taken up into the bloodstream. The blood and lymphatic systems including peripheral lymphoid organs are likely to be in contact with radon and its decay products.

The total radioactivity ingested by a radon-treated mouse in our experiments was about 640 Bq/kg in 4 weeks. Al-

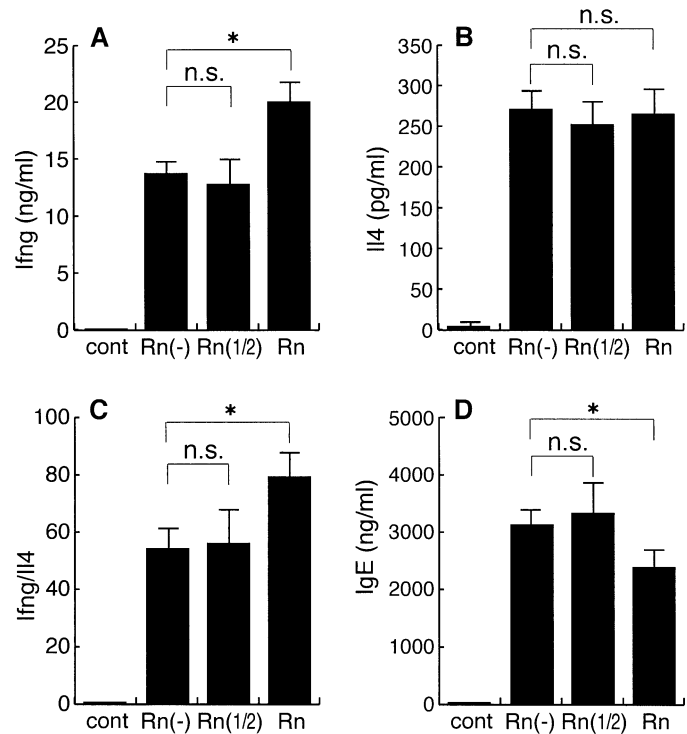


FIG. 3. Effect of radon on immunity balance in BALB/c mice immunized with DNP-Ascaris. Radon treatment was started 2 weeks prior to the immunization of mice and continued throughout the experiment. Cytokines produced by splenocytes from mice treated with water containing radon and plasma IgE were measured. Panel A: Ifng, panel B: Il4, panel C: the ratio Ifng/Il4, panel D: plasma IgE. Non-immunized control mice (cont) ($n = 4$), Rn(-) mice ($n = 8$), Rn(1/2) mice ($n = 7$), mice receiving radon ($n = 8$). n.s.: not significant. * $P < 0.05$. Data are the means $\pm$ SEM.

though a simple energy-based calculation gives a whole-body dose of 120 μ Gy, or 1.2 mSv with an RBE of 10, assuming that the absorbed dose in humans is 2.70 nGy/Bq of ingested radon (16), the actual dose in the irradiated cell is 0.1–0.5 Sv depending on the cellular location of the traversal. This indicates that energy deposition is highly localized in the tissues that come in contact with radon and its decay products.

In the present study, suppression of atopic dermatitis and tumor metastasis by small amounts of ingested radon was shown, though the effects were rather mild. In both experimental models, modulation of the immunity balance was found to have a significant impact on disease progression. In NC/Nga mice with atopic dermatitis, Ifng and Il12, which skew the balance toward Th1, have been shown to effectively ameliorate the disease when plasma IgE levels are low (6–8 μ g/ml at 10–12 weeks of age) (11), although when the levels were high (70–90 μ g/ml), these cytokines not only were ineffective but exacerbated the disease (12). The appearance of B cells committed to synthesize high amounts of IgE was suggested to be critical for the effect of these cytokines (12). This may explain our results showing that radon treatment was effective only when it was started before the atopic response was induced by picrylch-

loride. In the B16 tumor metastasis model, Th2-dominant immunity is established upon inoculation of B16 tumor cells (13). Il12 and Ifng exhibit anti-tumor effects through activation of NK and/or NKT cells (17–19).

Gamma radiation has been shown to increase Il12 and decrease Il10 at 0.075 Gy (20, 21). An increase in Ifng mRNA in lymphocytes after γ irradiation with 4 cGy was also shown (22). Enhancement of cytotoxic T-cell activity (23) and NK cell activity by γ radiation in mice (24, 25) has also been well documented. These reports imply the possible activation of cell-mediated immunity by a low dose of γ radiation. In the present study, we found increases in Ifng and the Ifng/Il4 ratio after treatment with radon in splenocytes from DNP-Ascaris-immunized mice. We also found that 0.5 Gy γ irradiation of mice induced an increase in Ifng and in the Ifng/Il4 ratio and an enhancement of NK and CTL activities in ICR mice.² In both cases, irradiated cells received similar equivalent doses of radiation. It may be suggested that low-dose irradiation of immune cells with either γ rays or α particles at ~ 0.5 Sv may modulate the immunity balance and enhance cell-mediated immunity.

Because some immunological responses depend on the antigen, type of response and mouse strain, as reported by Shanker *et al.* (26), simple generalizations cannot be made. For instance, we observed selective killing of B cells in the blood of γ -irradiated mice² but not in either the blood or spleen of radon-treated mice (data not shown). The mechanisms functioning in γ -irradiated mice and radon-treated mice may be different. In this study, different strains of mice were used to investigate the effects of radon in well-established disease models. As a result, there are limitations in mechanistic interpretation. Until the detailed mechanisms for the effect of radon are elucidated in the individual disease models, the possible involvement of modulation of immunity balance remains speculative.

Although we found that radon ingestion enhanced Ifng production in immunized mice, whether it was induced by direct irradiation of T cells or mediated by a signaling cascade through the bystander effect of α -particle radiation needs to be elucidated. The bystander effect in immune cells has been discussed largely in terms of the signaling cascades among functional cells (27–29), not the deleterious expansion observed in fibroblast cultures. Thus it is possible that irradiated cells signal the neighboring cells with cytokines or reactive oxygen species (30–32), including nitric oxide (27), to produce more Ifng.

The health effects of environmental radon at low concentrations have been questioned. There are epidemiological studies that have found a reduced cancer risk in the residents of high background radon areas (33). Immunological modulation by radon similar to that observed in our study may be involved. However, recent reports on cancer

risk among residents ingesting water with radon at a mean concentration of 130 Bq/liter found no increased risk of either stomach cancer or leukemia but also found no beneficial effects (34, 35). This may be because the effect of ingested radon at this concentration is not strong, as seen in our results, and many factors including timing and homeostatic balance would significantly influence the outcome. Thus we believe that only detailed mechanistic studies will answer the remaining questions.

To our knowledge, this is the first report on the beneficial effects of small amounts of radon on experimental disease models in mice. If adverse effects can be avoided, radon may be useful as a mild but effective immunological modulator.

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