Article

# Activity of Recombinant Human Interleukin-15 against Tumor Recurrence and Metastasis in Mice

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**Transplantable experimental tumor models were constructed to study the activities of recombinant human interleukin-15 (rhIL-15) against tumor recurrence and metastasis. The results showed that tumor nodule formation was retarded and tumor growth was inhibited in the subcutaneous tumor model of LA795 lung adenocarcinoma after treatment with rhIL-15, and the survival rate of T739 tumor-bearing mice treated with rhIL-15 was much higher than that of mice treated with either saline or with the same dose of rhIL-2. This indicats that rhIL-15 had better antitumor effect than rhIL-2 at the same dose level. In some rhIL-15 treated mice, the tumor cells inoculated subcutaneously were eradicated and there was no tumor formation even 138 days after tumor cell inoculation. The tumor-free mice were rechallenged with live tumor cells and no tumor reoccurred in the following two months in all of these mice, indicating that long-lasting antitumor systemic immunity developed. It was also shown that tumor recurrence and metastasis were inhibited markedly after treatment with rhIL-15, but not with the same dose of rhIL-2, in both subcutaneously and intravenously disseminated tumor models of LA795 lung adenocarcinoma. Simultaneously, the CTL and NK cell activities of the splenocytes obtained from tumor-bearing mice that had been treated with either rhIL-15 or rhIL-2 were both markedly enhanced. However, the enhancement of CTL and NK cell activities was more significant in rhIL-15 treated mice than that in rhIL-2 treated mice. This suggests that the anti-tumor effect of rhIL-15 *in vivo* was achieved by enhancing the CTL and NK cell activities in tumor immune response. *Cellular & Molecular Immunology*. 2008;5(3):189-196.**

**Key Words:** rhIL-15, LA795, transplantable experimental tumor model, recurrence, metastasis

## Introduction

Interleukin-15 (IL-15) is a regulative cytokine, produced by a variety of cell types, but not by T cells (1-3). IL-15 plays an important role in immune response and shares many functions with IL-2, for example, stimulating the proliferation of activated T cells (1, 4), NK cells (2, 5) and B cells, and inducing immunoglobulin synthesis by B cells stimulated by anti-IgM or CD40 ligand (6). In addition, IL-15 promotes the development of dendritic cells (7), activates human neutrophils (8) and induces the production of proinflammatory cytokines from macrophages (9). IL-15 acts as a bridge between innate and adaptive immunity because of its diverse roles in the immune system.

The antitumor roles of IL-15 have been demonstrated in several experimental tumor models. IL-15 prolonged the survival time of lymphoma-bearing mice (10) and suppressed pulmonary metastases established by intravenously (*i.v.*) disseminated sarcoma cells (11). When administered in combination with cyclophosphamide (CY), IL-15 acted as an adjuvant, markedly prolonged the survival time of CY treated mice bearing the intramusclary (*i.m.*) implanted 76-9 rhabdomyosarcoma (12), even induced permanent tumor regression and cured 32% of mice bearing established experimental pulmonary metastases of 76-9 rhabdomyosarcoma (13). Consecutive daily injections of IL-15 markedly inhibited tumor progression of melanoma with occasional curative effects. When used in combination with B78/IL-12 melanoma vaccine, IL-15 caused eradication of established tumors in all treated mice (14). In our previous studies, the antitumor activity of human IL-15 was also demonstrated through the application of IL-15 gene-modified tumor cells and the iniection of naked pcDNA3-IL-15 plasmid muscular into mice (15). However, to date, IL-15 used alone against tumors recurrence and metastasis is reported infrequently, although its inhibitory activity on tumor has been reported in the

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literatures. Therefore, *in vivo* activity of human IL-15 against tumor recurrence and metastasis was examined in this study.

## Materials and Methods

*Tumors and mice*

Murine LA795 lung adenocarcinoma cells (16, 17) were cultured at 37°C in a humidified atmosphere containing 5% $CO_2$ with RPMI 1640 supplemented with 10% FCS. Tumor cells were enzymatically digested in PBS containing 0.25% trypsin and washed, resuspended at desired concentrations in RPMI 1640, and used for injection into mice. Four to five weeks old T739 mice were obtained from the animal center of the Institute of Cancer Research, Chinese Academy of Medical Science & Cancer Hospital, Peking Union Medical College. The mice were fed under specific pathogen free conditions in our institute. All experiments began after the mice were observed for at least 2 weeks in the animal center.

*Reagents*

Lyophilized recombinant human IL-2 (specific activity: $9.0 \times 10^6$ units/mg protein) used as an active comparator, was kindly supplied by Read United Cross Pharmaceutical Co. Ltd., Beijing, China. The lyophilized recombinant human IL-15 (specific activity: $6.7 \times 10^6$ units/mg protein, the purity > 95%) was prepared in our laboratory.

*Establishment of subcutaneous transplantable tumor model*

Thirth-four male T739 mice at the age of twelve-week were assigned randomly into three groups, 12 mice in either the rhIL-15 or the rhIL-2 group and 10 mice in the control group. On day 0, the mice were injected subcutaneously (*s.c.*) into the right hind axilla with $1 \times 10^6$ LA795 lung adenocarcinoma cells. Three days following tumor cell inoculation, the mice began to receive daily *i.p.* injection with $4 \times 10^4$ units of rhIL-15 or rhIL-2 in 0.1 ml saline 5 days a week for 4 weeks (day 3 to day 30), and same volume of saline was given as control. Tumor growth and survival of tumor-bearing mice were observed every day. Tumor diameters were measured at the longest (a) and shortest (b) arms using calipers every 3 to 4 days, and the tumor size was calculated according to the formula: $ab^2/2$ ($mm^3$). Mice that became moribund due to tumors were killed, and tumors, lungs and livers were removed and kept for pathological examination. All sections were reviewed by the same pathologist with attention given to the metastatic lesions, as well as to the noninvolved parenchyma. A quantitative estimation was made of the number of lesions present in a given section. The differences of tumor size, survival time and metastasis between groups were analyzed to evaluate the antitumor efficacy of rhIL-15.

*Tumor recurrence*

To assess the efficacy of rhIL-15 against tumor recurrence at the early stage, subcutaneous inoculations of $5 \times 10^4$, $1 \times 10^5$ and $5 \times 10^5$ tumor cells were designed to imitate different tumor recurrence. Each inoculation dose group consisted of 26 seven-week female mice with 9 mice in the rhIL-15 and rhIL-2 group and 8 mice in control group, respectively. The main processes were illuminated as follows. Three days before tumor inoculation, the mice began to receive daily *i.p.* injections with $4 \times 10^4$ units of rhIL-15 or rhIL-2 in 0.1 ml saline 5 days a week for 12 weeks (day 3 to day 82), and the same volume of saline was given to the control group. On day 0, the mice were injected *s.c.* into the right hind back with tumor cells. All mice in an inoculation group were inoculated with the same cell suspension. Tumor growth and survival of tumor-bearing mice were observed every day. The mice that experienced complete tumor regression were rechallenged with $5 \times 10^4$ live LA795 lung adenocarcinoma cells 8 weeks after the end of treatment (on day 138).

*Tumor metastasis*

To assess the efficacy of rhIL-15 against tumor metastasis, $1 \times 10^5$ and $5 \times 10^5$ tumor cells were injected into T739 mice *via* tail vein to imitate tumor metastasis. Each group was consisted of 27 eight-week old female mice with 9 mice in the rhIL-15, rhIL-2 and the control group, respectively. The main processes were illuminated as following. Six days before tumor cell inoculation, the mice began to receive daily *i.p.* injections with $4 \times 10^4$ units of rhIL-15 or rhIL-2 in 0.1 ml saline 5 days a week for 7 weeks (day 6 to day 40), and the same volume of saline was given as control. On day 0, tumor cells were injected into mice *via* the tail vein. From then the survival of tumor-bearing mice was observed daily. Mice were sacrificed and autopsied 24 h (on day 41) after the last injection, and lungs were removed, insufflated by intratracheal injection of 15% India ink and placed in Fekete's solution. Metastatic tumors (visible as distinct white nodules on the black background of normal lung parenchyma) on all external lobar surfaces were counted (13, 18, 19).

*In vitro cytotoxicity assay*

A standard 4-h $^3$H-TdR-release assay was used to assess the cytotoxic activity of splenocytes. Briefly, splenocytes from rhIL-15 treated mice were isolated as described elsewhere (14, 20), depleted of red blood cells by Tris-ammonium chloride, and resuspended in RPMI 1640 as required cell concentration. The target cells used were YAC-1 (NK cell sensitive) and LA795 lung adenocarcinoma cells in NK cell and CTL assays, respectively. All target cells were maintained in RPMI 1640 supplemented with 10% FBS. The target cells were adjusted to $1 \times 10^5$ cells/ml and labeled with 5 μCi $^3$H-TdR/ml for 4 hours. Effector splenocytes and $^3$H-TdR-labeled target cells ($1 \times 10^4$ cells/well) were mixed in 96-well V-bottom plates at various E:T ratios, incubated for 4 hours at 37°C in 5% $CO_2$ atmosphere. The cells were harvested onto cellophane paper and the radioactivity was measured with a liquid scintillation counter. The percentage of specific lysis was calculated according to the formula: $[(a\text{-}c)/(b\text{-}c)] \times 100\%$, where $a$ is the test release cpm (incubation of target cells with splenocytes), and $b$ is the maximum release cpm (incubation of target cells with 2.5% Triton X-100), and $c$ is the spontaneous release cpm (incubation of target cells with media alone). Calculations were based on triplicate samples.

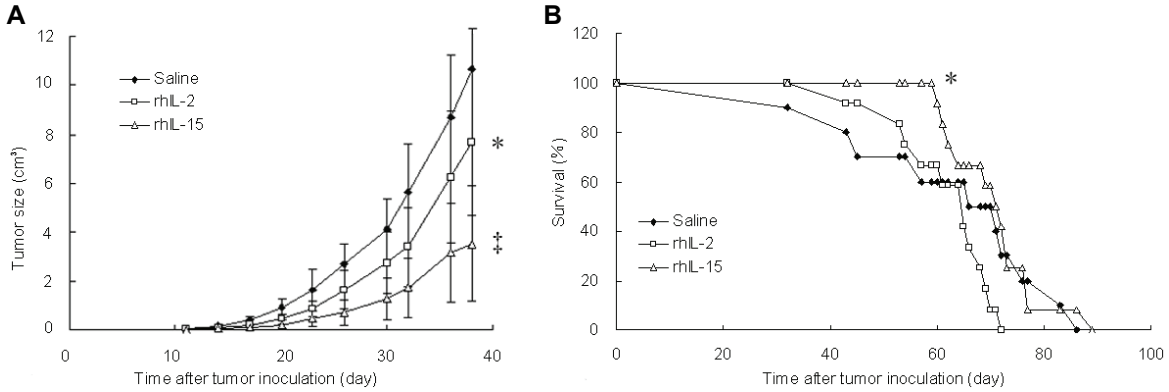


**Figure 1. RhIL-15 inhibited tumor growth *in vivo* and enhanced the survival of T739 mice bearing LA795 lung adenocarcinoma.** RhIL-15, rhIL-2 and control group consisted of 12, 12 and 10 mice, respectively. Mice were inoculated *s.c.* into the right hind axilla with $1 \times 10^6$ LA795 lung adenocarcinoma cells on day 0, and treated with rhIL-15, rhIL-2 or saline 5 days a week for 4 weeks (day 3 to day 30), respectively. (A) Tumor growth was recorded as mean tumor size ($cm^3$) ± SD every 3 to 4 days, and (B) the survival rate of each group was recorded every day. The tumor sizes of either rhIL-15 or rhIL-2 treated mice differed significantly from those of the control group ($^{\ddagger}p < 0.001$ and $^{*}p < 0.05$, respectively). On day 59, the survival rate of rhIL-15 group was higher than that of either the rhIL-2 or the control group ($^{*}p < 0.05$).

*Statistical analysis*

The two-tailed student's *t* test and $\chi^2$ test were used for the analysis of survival rate and tumor size between groups, respectively, whereby $p < 0.05$ indicated the significant difference.

## Results

*RhIL-15 inhibited the growth of murine LA795 lung adenocarcinoma more markedly than rhIL-2*

The antitumor efficacy of rhIL-15 was evaluated by tumor size, survival time and metastasis. Tumor growth was suppressed significantly by rhIL-15, whereas only a mild suppressive effect was observed with rhIL-2 in the subcutaneous transplantable experimental tumor model. Starting from day 11 and day 14, the tumor sizes in the mice treated with rhIL-15 ($p < 0.001$) and rhIL-2 ($p < 0.05$) were significantly smaller than those of control group, respectively. In addition, starting from day 14, the tumor sizes of rhIL-2 treated mice were larger than those of rhIL-15 treated mice ($p < 0.05$) (Figure 1A). Thirty-two days following the tumor cells inoculation, the mice began to die, and the survival rate of either the rhIL-15 or the rhIL-2 group was higher than that of the control group prior to day 59. On day 59 the survival rate of rhIL-15 group was 100%, higher than that of either rhIL-2 (66.7%, $p < 0.05$) or control group (60%, $p < 0.05$), as shown in Figure 1B. The maximum survival time of rhIL-15 treated mice was 89 days, longer than that of either rhIL-2 (72 days) or saline treated mice (86 days).

As shown in Table 1, there were 7 mice that showed tumor metastasis in rhIL-15, 8 in rhIL-2 and 7 in the control group,

**Table 1.** Tumor metastasis happened in tumor-bearing mice

| | Severity grades of metastasis | Control (N/n) | RhIL-2 (N/n) | RhIL-15 (N/n)) |
|---|---|---|---|---|
| Mice with lung metastasis | Grades I and II | 0/10 | 4/11 | 6/12 |
| | Grades III and IV | 4/10 | 2/11 | 0/12* |
| | Total | 4/10 | 6/11 | 6/12 |
| Mice with liver metastasis | Grades I and II | 1/9 | 1/10 | 3/11 |
| | Grades III and IV | 6/9 | 7/10 | 0/11$^{\dagger}$ |
| | Total | 7/9 | 8/10 | 3/11 |
| Mice with lymph node metastasis | Grades I and II | 0/10 | 0/11 | 2/12 |
| | Grades III and IV | 1/10 | 1/11 | 1/12 |
| | Total | 1/10 | 1/11 | 3/12 |

Note: Mice were inoculated *s.c.* into the right hind axilla with $1 \times 10^6$ LA795 lung adenocarcinoma cells on day 0, and treated with rhIL-15, rhIL-2 or saline 5 days a week for 4 weeks (day 3 to day 30), respectively. N is defined as the number of organs with tumor metastasis, and n is the number of organs examined. Tumor metastasis severity grades: grade I, only single or less than 5 small tumors in the full lung or liver; grade II, more than 5 small tumors in the full lung or liver; grade III, tumor is large, number not limited; grade IV, the full lung or liver lobe is occupied by tumor ($^{*}p < 0.05$ *vs* control, $^{\dagger}p < 0.01$ *vs* control).

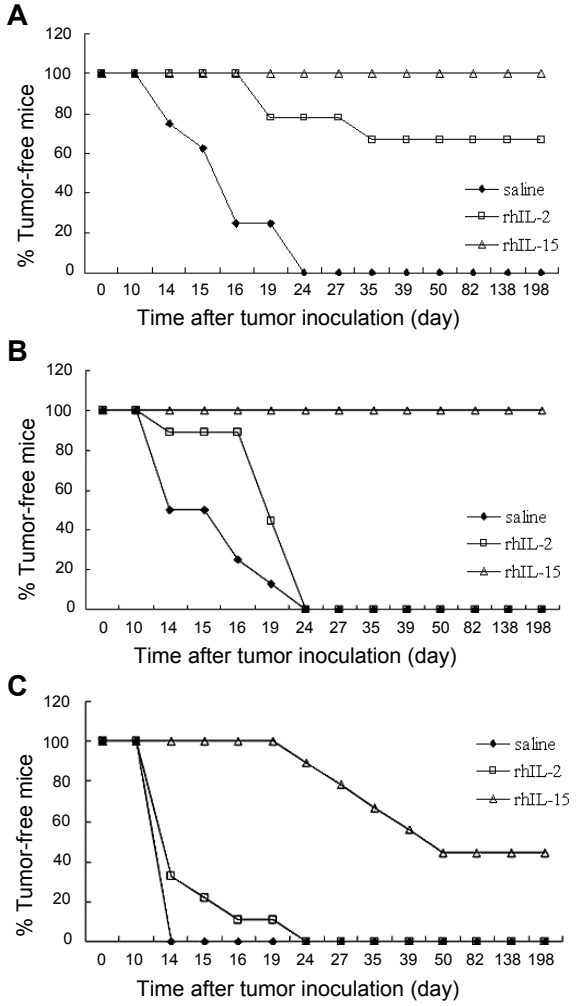


**Figure 2. RhIL-15 inhibited the tumor nodule formation of LA795 lung adenocarcinoma at early phase.** Mice were inoculated *s.c.* into the right hind back with various doses of LA795 lung adenocarcinoma cells on day 0. Mice were treated with rhIL-15, rhIL-2 or saline 5 days a week on day 3 to day 82. On day 138 following the tumor cell inoculation (i.e., 8 weeks after treatment was terminated), all of the tumor-free mice were rechallenged with $5\times10^4$ live LA795 lung adenocarcinoma cells by injecting *s.c.* into the contralateral hind back of the first time inoculation site. Percentages of tumor-free mice at the dose of $5 \times 10^4$ cells/mouse (A), $1 \times 10^5$ cells/mouse (B) and $5 \times 10^5$ cells/mouse (C) were observed every day. Each inoculation dose group consisted of 26 seven-week old female mice with 9 mice in the rhIL-15 and the rhIL-2 group and 8 mice in control group.

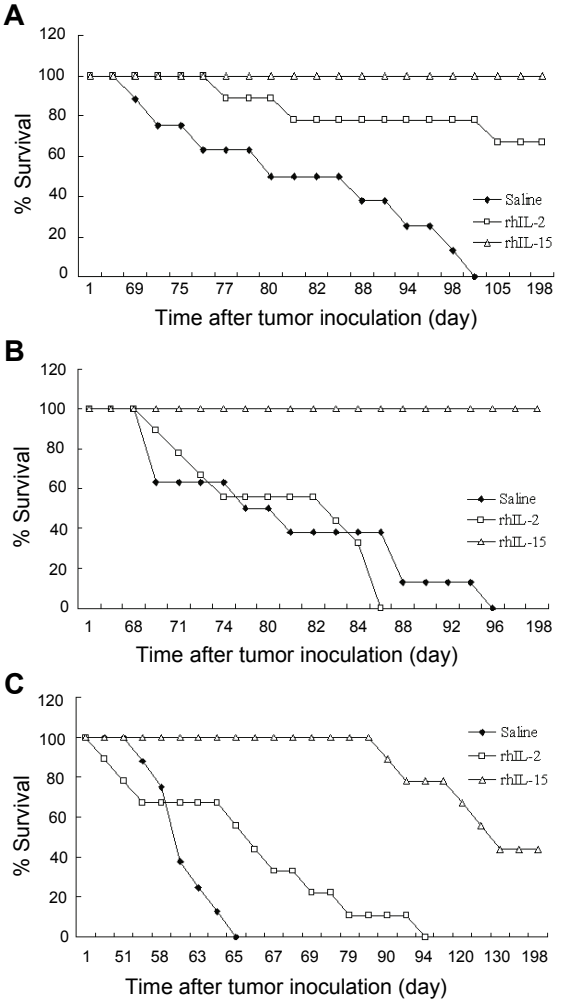


**Figure 3. RhIL-15 enhanced the survival rate of T739 mice inoculated murine LA795 lung adenocarcinoma.** Mice were injected *s.c.* into the right hind back with various doses of LA795 adenocarcinoma cells on day 0. Mice were treated with rhIL-15, rhIL-2 or saline 5 days a week on day 3 to day 82. On day 138 following the tumor cell inoculation (i.e., 8 weeks after treatment was terminated), all of the tumor-free mice were rechallenged with $5\times10^4$ live LA795 lung adenocarcinoma cells by injecting *s.c.* into the contralateral hind back of the first time inoculation site The survival rates at the dose of $5 \times 10^4$ cells/mouse (A), $1 \times 10^5$ cells/mouse (B) and $5 \times 10^5$ cells/mouse (C) were observed every day. Each inoculation dose group consisted of 26 seven-week old female mice with 9 mice in the rhIL-15 and the rhIL-2 group and 8 mice in control group.

respectively. Pulmonary metastasis was either grade I or II in the rhIL-15 treated mice, thereby significantly milder than that in the control group, which was either grade III or IV ($p < 0.05$). Pulmonary metastasis of the rhIL-2 treated mice was also milder than that of the control group, but there was no statistical significance. Metastasis in livers was similar to that in lungs among the three groups. In the rhIL-15 group, liver metastasis was either grade I or II, and significantly milder than that in the rhIL-2 and control group ($p < 0.01$), which was mostly grade III or IV. There was no significant difference for lymphatic metastasis among the three groups.

*RhIL-15 inhibited the recurrence of murine LA795 lung adenocarcinoma*

Tumor recurrence at early phase was imitated by *s.c.* inoculation into the right hind back of T739 mice with various doses of LA795 lung adenocarcinoma cells ($5 \times 10^4$, $1 \times 10^5$ and $5 \times 10^5$ cells/mouse). As shown in Figure 2, at

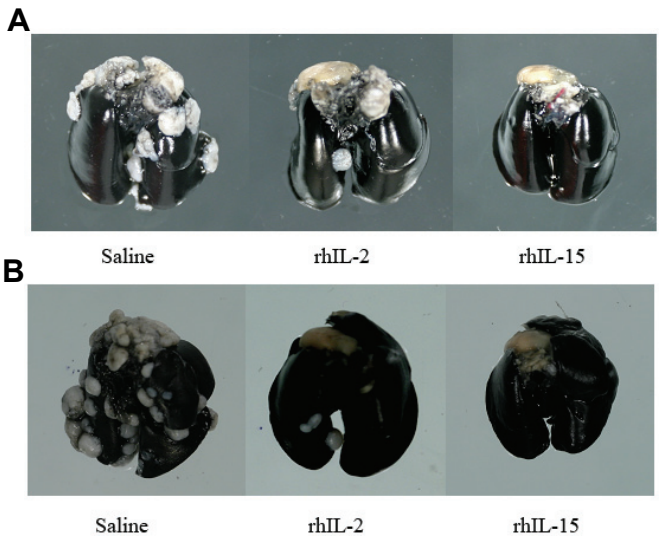


**Figure 4. RhIL-15 inhibited the pulmonary metastasis of murine LA795 lung adenocarcinoma.** Mice were injected intravenously with $1 \times 10^5$ or $5 \times 10^5$ LA795 lung adenocarcinoma cells on day 0. Mice were treated with rhIL-15, rhIL-2 or saline 5 days a week on day 6 to day 40. Mice were sacrificed and autopsied on day 41. Lungs were removed and infused with 15% India ink and bleached by Fekete's solution. The presence of metastatic tumors on the lung surface is shown above. Left, middle and right are representative lungs from the randomly selected mice treated with saline, rhIL-2 and rhIL-15, respectively. The number of metastatic tumors on the lung surface of the rhIL-15 treated mice is markedly less than those in the control group, $p < 0.01$. Each group contained 9 mice.

the dose of $5 \times 10^4$ cells/mouse, the first palpable tumor node was found on day 14, and palpable tumor nodes were found in all control mice on day 24. However, the first and the last palpable tumor nodes were found on day 19 and day 35, respectively, only 3 mice were found with tumor node formation in the rhIL-2 group and no mice formed palpable tumor node in the rhIL-15 group within 138 days following tumor cell inoculation. The final tumor-free percentage in the rhIL-15, rhIL-2 and control group was 100%, 67% and 0%, respectively (rhIL-15 *vs* control, $p < 0.001$). At the dose of $1 \times 10^5$ cells/mouse, the first palpable tumor node appeared on day 14 and palpable tumor nodes were found in all rhIL-2 or control mice on day 24, but no palpable tumor node was found in rhIL-15 treated mice even 138 days following tumor cell inoculation. The tumor-free percentage of rhIL-15 group (100%) was significantly higher than that of either the rhIL-2 (0%) or control group (0%). For the dose of $5 \times 10^5$ cells/mouse, palpable tumor node appeared on day 10 and palpable tumor nodes were found in all rhIL-2 or saline treated mice on day 14. However, the first palpable tumor node was not found until day 24 in the rhIL-15 group, and only 5 mice in total formed palpable tumor nodes eventually. The tumor-free percentage of the rhIL-15 group (44%) was clearly greater than that of both the rhIL-2 (0%) and control group (0%) ($p < 0.05$).

On day 138 (i.e., 8 weeks after treatment was terminated), all of the tumor-free mice were rechallenged with $5 \times 10^4$ live LA795 lung adenocarcinoma cells, by injecting *s.c.* into the contralateral hind back of the first time inoculation site. However, the LA795 lung adenocarcinoma cells were completely rejected and no tumor reoccurred in the following 2 months, indicating that long-lasting antitumor systemic immunity developed.

RhIL-15 not only inhibited and delayed the tumor node formation of LA795 lung adenocarcinoma at early phase, but also prolonged the survival time of the treated mice. As shown in Figure 3, at the dose of $5 \times 10^4$ cells/mouse, mice began to die 69 days after the tumor cell inoculation and the maximum survival time was 101 days in the control group. Mice began to die 77 days after the tumor cell inoculation in the rhIL-2 group, and only 6 mice remained alive in total until day 138. However, no mice in the rhIL-15 group died. The survival rate of the rhIL-15 group was higher than that of either the control ($p < 0.05$) or rhIL-2 group ($p > 0.05$). As the dose of $1 \times 10^5$ cells/mouse, mice began to die since day 69 in both the rhIL-2 and control group, and the survival time was 86 and 96 days, respectively. In the rhIL-15 group, no mice died. Therefore, both the survival rate and survival time of the rhIL-15 group were greater than those of the rhIL-2 or control group ($p < 0.001$). At the dose of $5 \times 10^5$ cells/mouse, mice died beginning on day 57 in the control group and day 44 in the rhIL-2 group, while the longest survival time was

**Table 2.** The number of mice developing tumor metastasis in each group

| Tumor cells inoculated | Treatment | Pulmonary metastasis (N/n) | Liver metastasis (N/n) | Other organ metastasis (N/n) | Tumor metastasis (N/n) |
|---|---|---|---|---|---|
| $1 \times 10^5$/mouse | saline | 9/9 | 4/9 | 4/9 | 9/9 |
| | rhIL-2 | 5/9* | 1/9 | 1/9 | 6/9 |
| | rhIL-15 | 4/9* | 0/9* | 0/9* | 4/9* |
| $5 \times 10^5$/mouse | saline | 9/9 | 1/9 | 2/9 | 9/9 |
| | rhIL-2 | 8/9 | 0/9 | 1/9 | 8/9 |
| | rhIL-15 | 6/9 | 1/9 | 0/9 | 6/9 |

Note: Mice were injected intravenously with $1 \times 10^5$ or $5 \times 10^5$ LA795 lung adenocarcinoma cells on day 0. Mice were treated with rhIL-15, rhIL-2 or saline 5 days a week from day 6 to day 40. "n" is the number of mice treated with rhIL-15, rhIL-2 or saline and "N" is the number of mice developing tumor metastasis in each group (*$p < 0.05$ *vs* control).

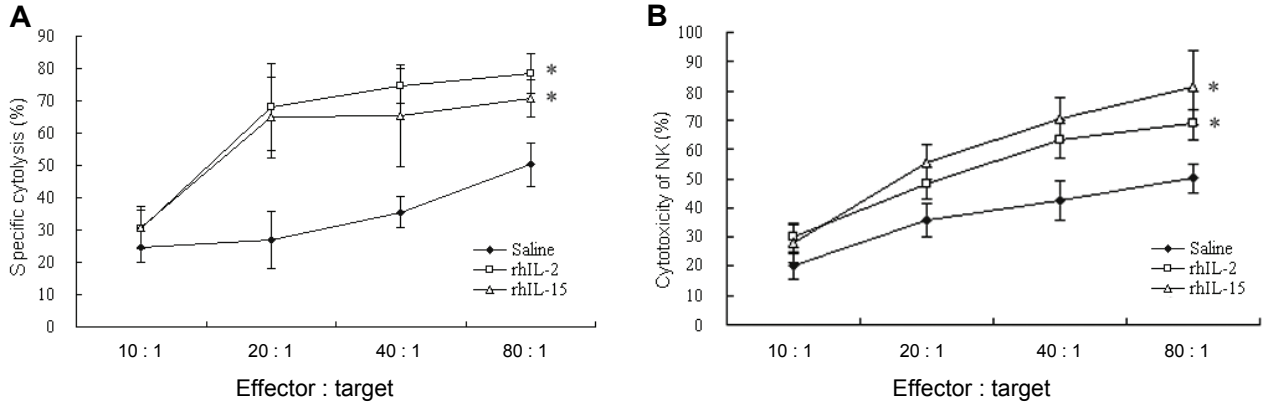


**Figure 5. NK/CTL mediated cytotoxicity of splenocytes isolated from the tumor-bearing mice.** Twenty-one female T739 mice at the age of six-week old were assigned randomly into three groups, i.e., rhIL-15, rhIL-2 and control, 7 mice in each group. On day 0, mice were injected *s.c.* into the right hind axilla with $5 \times 10^5$ LA795 lung adenocarcinoma cells. Ten days following tumor cell inoculation, mice began to receive daily *i.p.* injection with $4 \times 10^4$ units of rhIL-15 or rhIL-2 in 0.1 ml saline 5 days a week for 2 weeks (day 11 to day 22), and the same volume of saline was given to the control group. Mice were sacrificed 24 h after the last injection (day 23), and splenocytes were isolated and incubated with LA795 lung adenocarcinoma cells (A) or NK cell sensitive YAC-1 (B), respectively. Both the target cells were labeled with $^3$H-TdR. Values represent the mean ± SD of triplicate wells. Difference between the control and rhIL-15 group was significant, also between the control and rhIL-2 group for both NK and CTL activities (both $*p < 0.05$).

65 days in the control group and 94 days in the rhIL-2 group. In the rhIL-15 group, mice died starting day 90 and on day 138 four mice were still alive. The statistical analysis showed that both of the survival rate and survival time in the rhIL-15 group were significantly greater than those in the rhIL-2 ($p < 0.05$) and control group ($p < 0.01$).

*RhIL-15 inhibited the metastasis of murine LA795 lung adenocarcinoma*

Preliminary experiments indicated that $1 \times 10^5$ LA795 cells was the minimal dose that would result in development of pulmonary metastasis in all *i.v.* inoculated mice. The number of mice with tumor metastasis in each group was shown in Table 2. At the dose of $1 \times 10^5$, there were only 4 mice that developed lung metastasis and no mice that developed metastasis in the liver or other organs in the rhIL-15 group, which were less than those in the control group (both $p < 0.05$). In the rhIL-2 group the number of mice with lung metastasis, liver metastasis, and other metastasis was 5, 1 and 1, respectively, and with the similar results for the dosage of $5 \times 10^5$. The number of mice that developed metastasis in the lung or other organs and the number of mice bearing tumor metastasis in the rhIL-15 and rhIL-2 group were less than those in the control group.

In the mice inoculated with $1 \times 10^5$ or $5 \times 10^5$ LA795 lung adenocarcinoma cells and treated with either rhIL-15 or rhIL-2, both the number of mice that developed lung metastasis (Table 2) and the average number of metastatic tumors on the full lung surface were less than those in the control group (Figure 4). As shown in Figure 4, in general, there were more than 5 metastatic tumors on the full lung surface in the control group and 1~3 metastatic tumors in the rhIL-2 group. However, in the rhIL-15 group there was either one or no visible tumor on the lung surface. In addition, the metastatic tumor on the lung surface found in the rhIL-15 group was smaller than those in the rhIL-2 and control group.

*Cytotoxicity assay*

To determine whether the increased antitumor activity of rhIL-15 treated mice was associated with increased cytotoxicity, splenic lymphocytes were tested for cytotoxicity in a standard 4-h $^3$H-TdR-release assay. The cytotoxicity of splenic lymphocytes on day 23 was shown in Figure 5. The splenic lymphocytes isolated from rhIL-15 treated mice were highly cytotoxic against NK cell-sensitive targets (YAC-1) and LA795 cells. Enhancements of splenocyte cytotoxicity to the target YAC-1 and LA795 cells were significant in both rhIL-15 and rhIL-2 groups, respectively. Thus, it was suggested that enhancing host CTL/NK cytotoxicity might be one of the mechanisms contributing to antitumor effects of rhIL-15.

## Discussion

The present study provides the evidence that IL-15 administration played an important role in tumor therapy. Since 1998, the antitumor effect of IL-15 has been generally acknowledged (10-14, 21-23), and it has been recognized as a more promising cytokine than IL-2, with the potential for application in tumor therapy and vaccine design (23) since IL-15 is more potent than IL-2 in tumor therapy with greater therapeutic index (11). IL-15 can also obviously decrease the toxicity of chemotherapy to the gastrointestinal tract and can increase the maximum tolerant dose of chemotherapeutic drug in rat colon cancer model (24). In addition, the cytokines IL-15 and IL-7 are important in memory T cell homeostasis (15). Therefore, IL-15 may be a candidate

cytokine for broad-spectrum of tumors, especially for those unsuccessfully treated by IL-2.

The available data show that several mechanisms contribute to the overall therapeutic effects of IL-15. Firstly, IL-15 is a strong activator of NK cells and facilitates the maturation and differentiation of cytotoxic NK cells (25, 26). In our previous studies, the NK cytotoxicity of the splenocytes obtained from tumor-bearing mice treated with either $^{60}$Co irradiated IL-15 gene-transduced LA795 tumor cells or naked pcDNA3-IL-15 plasmid was markedly enhanced (27), which is confirmed by the present study. Secondly, IL-15 promotes MHC-restricted antitumor response, including the development of dendritic cells, which induces potent Th1 and Tc1 responses *in vivo* (7). IL-15 also induces the generation of cytolytic effector T cells (1, 28), and helps maintaining memory $CD8^+$ T cells (29). The CTL cytotoxicity of the splenocytes obtained from tumor-bearing mice treated with IL-15 was markedly enhanced in the present study. IL-15 may enhance the cross-priming of $CD4^+$ T and $CD8^+$ T cells to increase the CTL cytotoxicity, or selectively stimulate the proliferation of memory phenotype $CD44^{hi}CD8^+$ T cells and promote the memory CTL function. It was also manifested that the inhibitory effect of IL-15 on tumor growth was at least T cell dependent and the $CD8^+$ CTL might be a major effector in BALB/c nude mice in our previous study (27). Finally, IL-15 can inhibit the development of tumor *via* nonimmune mechanisms, such as inducing the expression of perforin and granzymes in murine lymphocytes (30) and activating human PBL for perforin-mediated cytolysis of melanoma and lung cancer (31, 32).

In addition, the interaction of NK cells with tumor-specific αβ or γδ T lymphocytes was necessary for successful IL-15 therapy. The IL-15 therapy resulted in increased levels of $NK1.1^+$/$LGL\text{-}1^+$ cells, and $CD8^+$/$CD44^+$ T cells in PBL (13). In view of the reports that IL-15 may activate macrophages, NK cells, and polymorphonuclear precursor at the tumor site (8, 9, 25, 26), the administration of IL-15 clearly has the potential to accentuate the antitumor roles that each or all of these cells play. IL-15 facilitates the production of TNF-α and IFN-γ from T and NK cells (10, 33, 34), and this in turn may augment T cell immune responses at the tumor site, including the generation and activation of CTL and LAK cells. Probably, the increased level of IFN-γ was also responsible for augmented tumoricidal activity of macrophages in mice treated with IL-15. The importance of macrophages was also reported in the antitumor effect of IL-15 (35). Apart from stimulation of IFN-γ production, the therapy of IL-15 acted on other mechanisms that could be significant to the overall antitumor effect. IFN-γ-independent antitumor effect of IL-15 was mediated by $CD8^+$ T cells and involved secondary secreted chemokines such as MIP-2 and MCP-1 as well as cytokines TNF-α and GM-CSF (36).

Apart from the effects on immune cells, IL-15 down-regulates the expression of MHC class I molecules on murine LA795 lung adenocarcinoma and human PG lung squamous carcinoma cells, and releases the inhibition of NK and a few $CD8^+$ T cells, due to the interaction between inhibitory receptors (KIRs) and low expressed class I molecules (27). Therefore, the tumor cells expressing low levels of MHC class I molecules could be more sensitive to NK cells, and could be more easily to be killed by NK cells.

Presumably, at least some of the above mentioned mechanisms could be responsible for the development of cytotoxic effectors against LA795 tumor cells in mice treated with IL-15.

Tumor metastasis process includes that tumor cells isolate from the primary tumor focal, adhere to the adjacent microvessel endothelial cells and invade blood vessels more via the blood stream, disseminate and grow in the distant target tissues. During the infiltration and metastasis progress, tumor cells interact with extracellular matrix and host cells including immune cells (37). The inhibition of tumor recurrence and metastasis by therapy of IL-15 could be contributed to metastasized tumor cells being killed by immune cells during the infiltration and metastasis progress.

It has been reported that IL-15 combined with cyclophosphane can inhibit tumor metastasis in the established transplantable lung metastatic tumor model of 76-9 rhabdomyosarcoma. The tumor metastasis in CY + IL-15 treated tumor-bearing mice was fewer and lighter than those treated with CY alone (13). In the present study, the tumor metastasis happened later and mostly milder in the IL-15 treated tumor-bearing mice, compared with IL-2 treated and control tumor-bearing mice, in the subcutaneous transplantable experimental tumor model of murine LA795 lung adenocarcinoma. The same results were observed in the *i.v.* disseminated tumor model of LA795 lung adenocarcinoma. The administration of IL-15 effectively inhibited the tumor metastasis, and the frequencies of metastasis in lung, liver and other organs of IL-15 treated mice. All of those were lower than those in the control and IL-2 group. Therefore, these results indicated directly that IL-15 therapy could inhibit the tumor metastasis.

The efficacy of IL-15 against tumor recurrence and metastasis in mice provided the stimulus to explore the application of IL-15 to human. However, the use of exogenous IL-15 in patients is worth further exploration.

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