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Synergistic effect of interleukin-15 and interleukin-12 on antitumor activity in a murine malignant pleurisy model

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Abstract Interleukin(IL)-15, which uses IL-2 receptor (R) β and γ chains for signal transduction, shares many of the biological activities of IL-2. We examined the effects of exogenous IL-15 on protection in a murine malignant pleurisy model using BALB/c mice and syngeneic MethA fibrosarcoma (MethA). Intrapleural administration of IL-15 significantly prolonged the survival time of mice after an intrapleural inoculation of MethA, whereas the same dose of IL-2 did not. The in vivo antitumor effect of IL-15 was synergistically enhanced by additive administration of IL-12. Combination therapy of IL-15 and IL-12 protected mice from death from bloody pleural fluid. Such treatment induced marked increases in the number of $CD3^{-}IL\text{-}2R\beta^{+}$ cells corresponding to natural killer (NK) cells and the production of interferon γ (IFNγ) by T cells in the thoracic exudate cells (TEC). Administration of anti-IFNγ mAb partly inhibited the protective effect of a combination of IL-15 and IL-12. A tumor-neutralizing (Winn) assay revealed that the antitumor activity of effector cells in the TEC was abrogated by treatment with anti-CD8 mAb or anti-asialoGM1 Ab plus complement. Thus, treatment with IL-15 in combination with IL-12 may enhance the activities of NK and $CD8^{+}$ T cells in the TEC, providing strong antitumor activity against the malignant pleurisy. These results suggest that IL-15 together with IL-12 may have potential for the immunotherapy of some types of malignant pleurisy.

Key words IL-15 · IL-12 · MethA · Malignant pleurisy

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Introduction

Interleukin-15 (IL-15) is a novel 15-kDa cytokine [3, 13] that uses the β and γ chains of the IL-2 receptor (R) for signal transduction and shares many of the biological activities of IL-2 including induction of the proliferation of phytohemagglutinin-stimulated normal peripheral blood mononuclear cells (PBMC), natural killer (NK) cells and B cells [2, 3, 6, 13], and generation of cytotoxic T lymphocytes (CTL) and lymphokine-activated killer (LAK) cells in the PBMC in vitro [2, 3, 6, 13]. γδ T cells are stimulated to produce interferon γ (IFNγ) in response to exogenous IL-15 or IL-15 derived from infected macrophages [14, 26, 27, 31]. The memory phenotype of $CD8^{+}$ T cells has also been shown to respond preferentially to exogenous IL-15 [34]. Mice genetically lacking IL-15Rα are deficient in NK cells, γδ T cell receptor (TCR) intestinal intraepithelial lymphocytes and the memory phenotype of $CD8^{+}$ T cells [16]. Taken together, these characteristics make IL-15 important for development and maintenance of NK cells, a significant fraction of intestinal intraepithelial lymphocytes and the memory phenotype of $CD8^{+}$ T cells. Several reports based on murine models have suggested that IL-15 has antitumor effects. The daily administration of IL-15 prolonged the period of remission induced by cyclophosphamide in mice bearing rhabdomyosarcoma [15]. IL-15 is active in suppressing pulmonary metastasis in a murine model, albeit less active than IL-2 [10]. Thus, IL-15 may exert antitumor effects and be useful for cancer immunotherapy.

IL-12 is a heterodimer cytokine composed of a constitutively expressed 35-kDa (p35) subunit and an inducible 40-kDa (p40) subunit [30], which enhances the activity of NK cells [12], promotes development of Th1

cells and cytotoxic T cells [33] and enhances IFNγ production [22]. IL-12 has been shown to have potent antitumor and anti-metastatic activity in a number of tumor models including carcinoma, sarcoma, and melanoma [5, 8, 22, 24, 33]. Thus, IL-12 exerts its antitumor effects through its abilities to increase the lytic activities of NK cells, to enhance specific CTL responses [33], to induce IFNγ production by both resting and activated NK and T cells[22], and to stimulate Th1 cells that are associated with cellular immunity [8]. Recently, IL-15 has been found to up-regulate IL-12 Rβ1 in resting PBMC, which are important for production of IFNγ and proliferation of NK and Th1 cells [32]. Takeuchi et al. reported that IL-15 and IL-12 had additive effects on the induction of killer activity against a small-cell lung cancer cell line [29]. Taken together, these results imply that IL-15 may synergize with IL-12 to provide strong resistance against tumor.

In the present study, we examined the antitumor effects of exogenous IL-15 and IL-12 in a malignant pleurisy model using BALB/c mice and syngeneic MethA fibrosarcoma (MethA). Intrapleural administration of IL-15 in combination with IL-12 was most effective both for inducing the disappearance of exudate fluid in the thoracic cavity and in prolonging the survival of mice inoculated intrapleurally (i.pl.) with tumor. Combination cancer immunotherapy using IL-15 and IL-12 may be useful in at least some cases of malignant pleurisy.

Materials and methods

Animals and tumor cells

Inbred specific-pathogen-free male BALB/c mice 7–9 weeks old were purchased from SLC (Hamamatsu, Japan) and housed in a sterile, isolated room routinely screened for murine pathogens. MethA, a methylcholanthrene-induced fibrosarcoma syngeneic in the BALB/c mouse cell line, was maintained in ascitic form by weekly i.p. passages in BALB/c mice. Mice (ten mice per group) were inoculated i.pl. with MethA at a dose of 1×10^5 cells/mouse on day 0 as reported previously [17–19]. The animals were treated in compliance with institutional guidelines.

In vivo administration of mAb

Anti-IFNγ mAb (Genezyme) and isotype control Ab (rat IgG; Inter-Cell Technologies Inc., Hopewell, N.J.) were injected i.pl. on days 2 and 4 after inoculation of MethA at a dose of 20 μg mouse^{-1} day^{-1}.

In vivo administration of cytokines

Murine recombinat (r)IL-12, human rIL-15 and human rIL-2 were purchased from Genzyme (Cambridge, Mass). The purified recombinant cytokine was dissolved in phosphate-buffered saline (PBS) containing 5% normal mouse serum (vehicle). Samples of 100 μl solution containing different amounts of recombinant cytokines were administered i.pl. to BALB/c mice on days 0, 3, and 6 after inoculation of MethA. The same amount of PBS containing 5% normal mouse serum was used as control and administered i.pl. to BALB/c mice with the same schedule and procedure.

Preparation of thoracic exudate cells (TEC)

Thoracic exudate fluid was collected after the final injection of each treatment by washing the thoracic cavity of the mice with 2.5 ml Hanks' balanced salt solution; then the cells were allowed to adhere to plastic tissue-culture plates at 5×10^6/plate, incubated at 37 °C in complete RPMI medium (2 mM glutamine, 100 U/ml each of penicillin 6-potassium and streptomycin sulfate, 10% FCS, and 50 μM 2-mercaptoethanol; Life Technologies, Grand Island, N.Y.) and at 37 °C in 5% CO_2 for 2 h before being thoroughly rinsed with warm medium. The adherent fraction was then collected by scraping with a disposable cell scraper; nonadherent cells were retained separately and centrifuged through a 40%/50%/70% Percoll (Pharmacia, Uppsala, Sweden) gradient at 600*g* at 20 °C for 20 min. Cells were obtained at the 50%/70% interface, washed and used for the experiments as effector T cells. The number of viable cells was counted by trypan blue staining. The total number of TEC was counted in a hemocytometer. A cytocentrifuge preparation was made with the TEC suspension by staining with May-Grünwald and Giemsa solutions and examined microscopically. A differential cell count was performed by counting more than 100 cells and the percentage of each cell type calculated.

Antibodies and flow-cytometric analysis

All cell preparations suspended in PBS containing 2.5% Nu-serum and 0.1% NaN_3 were stained with the appropriate mAb at 4 °C for 30 min. The mAb used in these experiments were as follows: fluorescein-isothiocyanate(FITC)-conjugated anti-TCRαβ mAb, phycoerythrin(PE)-conjugated anti-TCRγδ mAb, FITC-conjugated anti-CD3, PE-conjugated anti-CD8 mAb (anti-Lyt2), biotin-conjugated anti-CD4 mAb (anti-L3T4), FITC-conjugated anti-CD122/IL-2Rβ (TMβ1), biotin-conjugated anti-Gr1, and PE-conjugated anti-Mac-1, all from PharMingen (San Diego, calif.). RED-613-conjugated streptavidin was from Gibco BRL (Gaithersburg, Md.). The surface antigens of cells were identified by using various mAb in conjunction with two- to three-color staining. The cells were analyzed by FACScan (Becton Dickinson) using the Lysis II program. The optimal concentrations of these mAb for staining were determined in preliminary experiments.

Stimulation of effector cells by plate-bound anti-CD3 mAb

Wells of microplates were coated with anti-CD3 mAb (10 μg/ml) at 4 °C overnight. After removal of unbound mAb, 2×10^5 effector T cells were added to each well and cultured in RPMI-1640 medium supplemented with 10% FCS, 100 U/ml each of penicillin 6-potassium and streptomycin and 50 μM 2-mercaptoethanol at 37 °C in 5% CO_2 for 24 h. The supernatants were used for the detection of cytokine (IFNγ, IL-4) production by an enzyme-linked immunosorbent assay kit (Amersham, Buckinghamshire, UK).

Winn assay and treatment with Ab and complement

Anti-CD4 (anti-L3T4), and anti-CD8 (anti-Lyt2.2) mAb in ascites (Cedarlane Laboratories Ltd., Ontario, Canada) were used, each at final dilutions of 1:100. Polyclonal rabbit anti-asialoGM1 Ab (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used at a final dilution of 1:50. As a complement source, Low-Tox-M rabbit complement (Cedarlane Laboratories Ltd.) was used at a final dilution of 1:10. Non-adherent TEC of mice bearing i.pl. tumors, injected with a combination of rIL-15 and rIL-12, were collected on day 7 and suspended at 2×10^7 cells/ml in RPMI-1640/10% FCS. Ab and complement were simultaneously added to the cell suspension and incubated at 37 °C for 2 h. After the incubation, the cells were collected and washed twice with serum-free RPMI-1640 medium. After the treatment with Ab plus complement, the number of viable cells was determined by the trypan blue dye exclusion test, and this number was used as the number of effector cells. Samples containing 1×10^5 MethA cells were inoculated s.c. alone

or were co-injected with 5×10^6 effector cells treated with various Ab into normal BALB/c mice (seven mice per group). The tumor diameter was measured by calipers, and tumor volume was approximately determined from the following formula:

$$\text{Tumor volume } (\text{mm}^3) = \frac{[\text{short diameter (mm)}]^2 \times [\text{long diameter (mm)}]}{2}$$

Statistical analysis

The statistical significance of the survival rate was determined by the generation of Kaplan-Meier cumulative-hazard plots and log-rank (Mantel-Cox) analysis. Other data (i.e. tumor size, cell count, cell population) were analyzed using an analysis of variance (ANOVA) model controlling for treatment, experiment and the potential for an experiment by treatment interaction. $P < 0.05$ was taken as the level of significance. Analyses were completed using Stat-View 4.5 software (Abacus Concepts, Berkeley, calif.) and Power Macintosh 7200 computers (Apple Computer, Cupertino, calif.)

Results

In vivo antitumor effects of recombinant IL-15 and synergic effects of IL-12 and IL-15 in mice inoculated i.pl. with MethA

Employing a malignant pleurisy model [17–19], we investigated the effect of intrapleural administration of IL-15 on survival of BALB/c mice inoculated i.pl. with MethA. As in previously reported work [17], all the mice in the control inoculated with PBS died within 10 days. We also observed an increase in the bloody effusion in the thoracic cavity from day 6 after inoculation. Intrapleural administration with more than 100 ng rIL-15 each time on days 0, 3, and 6 after i.pl. injection of MethA significantly enhanced the mean survival time (21.2 ± 4.5 days $n = 10$) as compared with control mice (10.8 ± 1.5 days, $n = 10$) (Fig. 1). Two out of ten mice were free of tumor in the groups treated with rIL-15, whereas all the mice in control groups died of bloody pleural fluid. Treatment with 50 ng, 100 ng or 200 ng IL-2 every 3 days had only a marginal antitumor effect, less than that of rIL-15 (Fig. 1, data not shown).

We next examined the same treatment using rIL-12, and intrapleural treatment of mice with 100 ng rIL-12/dose on days 0, 3, and 6 after an intrapleural injection of MethA only marginally prolonged the mean survival time but did not increase the survival rate of mice inoculated i.pl. with MethA (Fig. 2). In vivo treatment with 200 ng rIL-12 exhibited no effect on the survival time and rate (data not shown). Notably, rIL-15, when used in combination with rIL-12, markedly enhanced the mean survival time and rate of the tumor-bearing mice (Fig. 2). Six out of ten mice were free of tumor in the groups where each mouse was treated with 100 ng rIL-15 and rIL-12 i.pl. simultaneously, whereas all mice in the respective control groups died of bloody pleural fluid. In a separate experiment, counting of tumor cells in the thoracic cavity of mice after intrapleural lavage and staining 7 days after tumor challenge showed that intrapleural treatment with rIL-15 and rIL-12 decreased the number of tumor cells in the intrapleural cavity to about 20% of control levels (data not shown). The possibility that rIL-15 or rIL-12 had a direct cytotoxic or cytostatic effect was ruled out by the finding that a concentration of rIL-15 or rIL-12 as high as 100 μg/ml had no effect on the growth of MethA cells in vitro (data not shown). No visible side-effects, such as weight loss or

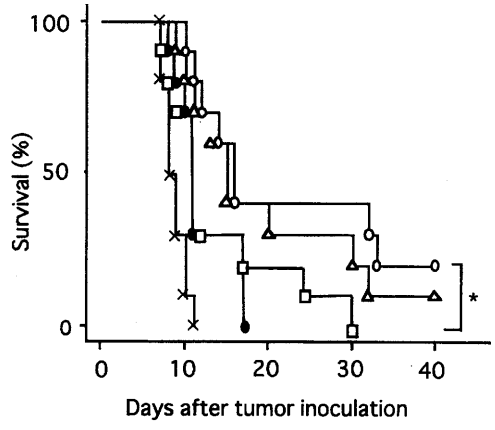

Fig. 1 The effect of recombinant interleukin-15 (rIL-15) or rIL-2 on the survival rate after intrapleural (i.pl.) administration of MethA. Five groups of BALB/c mice inoculated i.pl. with 1×10^5 MethA cells (ten mice per group) were injected i.pl. with 50 ng (□), 100 ng (○), or 200 ng (△) rIL-15, (●) 100 ng rIL-2 or phosphate-buffered saline (PBS; ×) on days 0, 3, and 6 after MethA inoculation. $^*P < 0.05$ compared to the values for control mice treated with PBS (by log-rank test)

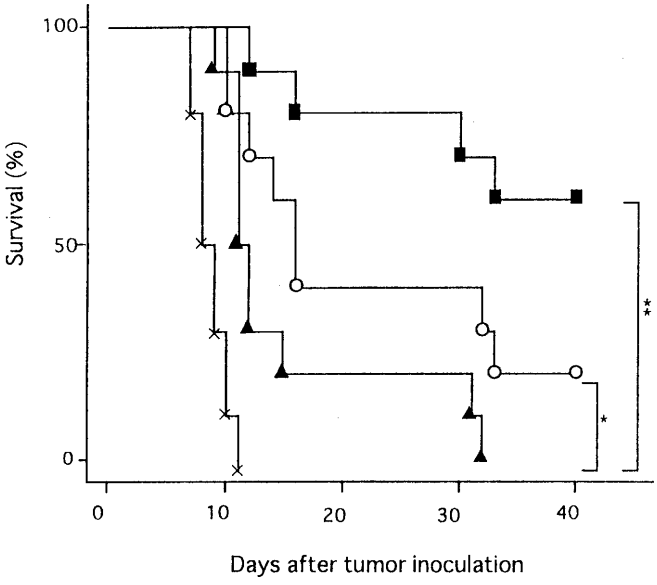

Fig. 2 The effect of rIL-15, rIL-12 or a combination of rIL-15 and rIL-12 on the survival rate after i.pl. administration of MethA. Four groups of BALB/c mice inoculated i.pl. with 1×10^5 MethA cells (ten mice per group) were injected i.pl. with 100 ng rIL-15 (○), rIL-12 (▲), rIL-15 + rIL-12 (■), or PBS (×) on days 0, 3 and 6 after inoculation of MethA. $^*P < 0.05$, $^{**}P < 0.01$ significantly different from the values for control mice treated with PBS (by log-rank test)

diarrhea, were observed in mice receiving short-term treatment with rIL-15 and/or rIL-12 at the doses used in this study.

Effects of rIL-15 and rIL-12 on cellular responses in mice inoculated i.pl. with MethA

Focusing on the change observed on day 7 after MethA inoculation, we investigated the in vivo effect of treatment with rIL-15 and rIL-12 on the characterization of the TEC to elucidate the mechanisms of these antitumor effects. The numbers of TEC significantly increased in all groups treated with rIL-15 or with a combination of rIL-15 and rIL-12 but not in mice treated with rIL-12 (Table 1). No significant difference was found in the proportion of $CD3^+$, $CD4^+$, or $CD8^+$ cells in the TEC of each group. In the treatment with rIL-15 and rIL-12 combined, the $CD3^-$ IL-$2R^+$ population representing NK cells, significantly increased (Table 1).

We next examined the cytokine production in vivo and in vitro by non-adherent effector cells in i.pl. MethA-bearing mice. We confirmed a marked increase in IFNγ levels in thoracic exudate fluids in mice treated with a combination of rIL-15 and rIL-12 on day 7 after MethA inoculation (852 ± 155 pg/ml, $n = 5$), while no appreciable level of IFNγ was found in PBS-treated mice. To clarify the source of such augmentation, we investigated the level of IFNγ after the in vitro proliferation of T cells in the TEC, as described in Materials and methods. We found significantly greater levels of IFNγ production by thoracic exudate T cells of mice treated with rIL-12 alone or with a combination of rIL-15 and rIL-12 but not in mice treated with rIL-15 alone (Fig. 3).

Table 1 Characterization of thoracic exudate cells (TEC) from MethA-bearing mice treated with recombinant interleukin-15 (rIL-15), rIL-12 or a combination of rIL-15 and rIL-12. The results show the percentages of positive cells among the total number of infiltrating leukocytes. Mice (ten mice per group) were i.pl. inoculated with MethA on day 0 and treated with either 100 ng rIL-15, 100 ng rIL-12 or a combination of 100 ng rIL-15 and rIL-12 on days 0, 3, and 6 after inoculation of MethA. TEC were collected on day 7 and stained with mAb

Antigen	Positive cells (%)			
	Control	rIL-15	rIL-12	rIL-15 + rIL-12
$Macl^+$	33 ± 9	29 ± 8	26 ± 11	24 ± 8
$Gr\text{-}1^+$	37 ± 12	38 ± 7	33 ± 5	29 ± 9
$CD3^+$	23 ± 6	29 ± 12	35 ± 10	33 ± 14
$CD4^+$	12 ± 3	14 ± 5	16 ± 4	15 ± 5
$CD8^+$	7 ± 2	10 ± 7	11 ± 6	10 ± 8
$CD3^-$ IL2R β^+	2 ± 1	5 ± 3	5 ± 2	9 ± 2*
10^{-5} × total cell number[a]	85 ± 14	130 ± 21*	119 ± 24	140 ± 22*

*$P < 0.05$ significantly different from control values by ANOVA
[a] Mean number for ten mice in each group ± SD

IFNγ is important in the antitumor effect of rIL-15 and rIL-12 in mice inoculated i.pl. with MethA

To elucidate the direct roles of IFNγ in the antitumor activity of a combination of rIL-12 and rIL-15, anti-IFNγ mAb were injected into MethA-bearing mice treated with a combination of rIL-15 and rIL-12. As shown in Fig. 4, in vivo administration of anti-IFNγ

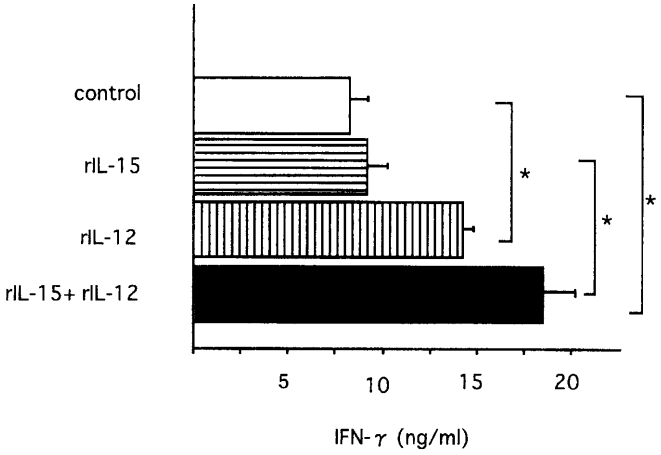

Fig. 3 In vitro interferon γ (IFNγ) production of thoracic exudate T cells (TEC). Non-adherent TEC in MethA-bearing mice, treated with either PBS, 100 ng rIL-15, 100 ng rIL-12, or a combination of 100 ng each of rIL-15 and rIL-12 on days 0, 3, and 6, were collected after the final treatment. The cells (3×10^5/well) were cultured in plates coated with anti-CD3 mAb (10 μg/ml) for 48 h, and the IFNγ content in the supernatants was measured by enzyme-linked immunosorbent assay. Results are presented as the means ± SD of five mice of each group. *$P < 0.05$ significantly different from the values for control by ANOVA

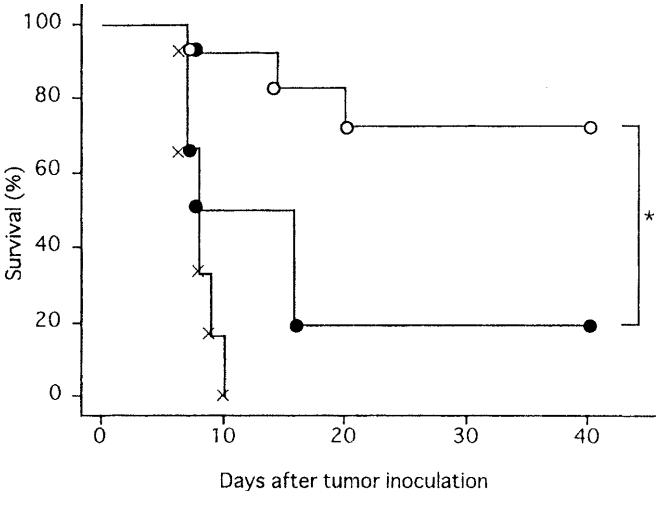

Fig. 4 The effects of in vivo administration of anti-IFNγ mAb on antitumor activity of a combination of rIL-12 and rIL-15 . BALB/c mice were inoculated i.pl. with PBS (×) or a combination of 100 ng each of rIL-12 and rIL-15 on days 0, 3, and 6 after MethA inoculation. Anti-IFNγ mAb (●) or isotype control Ab (○) (20 μg/mouse/day) was injected i.pl. into rIL-15 + rIL-12-treated mice on days 2 and 4 after inoculation of MethA (ten mice per group). *$P < 0.05$ different from values for mice treated with isotype control Ab (by log-rank test)

mAb partly but significantly inhibited the protective effects of a combination of rIL-15 and rIL-12. These results suggest that IFNγ is involved in the antitumor effect of a combination of rIL-15 and rIL-12 in the malignant pleurisy model.

Effector cells in the antitumor effect of rIL-15 and rIL-12 in mice inoculated i.pl. with MethA

To detect and characterize the phenotypes of the intrapleural antitumor effector cells against MethA in the tumor-bearing mice treated with a combination of rIL-15 and rIL-12, we performed a Winn assay as described in Materials and methods. The effector activity was inhibited by treatment with anti-CD8mAb or anti-asialoGM1 Ab plus complement, though not completely, whereas anti-CD4 mAb did not abrogate the effector activity (Fig. 5). We suppose that at least two kinds of anti-MethA effector cells with different surface antigens, positive for CD8 and asialoGM1, were induced in the MethA-bearing BALB/c mice injected with rIL-15 together with rIL-12.

Discussion

In the present study, we obtained the first evidence that IL-15 in synergy with IL-12 exhibits a strong antitumor effect in a malignant pleurisy model. The effector activity for treatment with a combination of IL-15 and IL-12 is dependent on at least IFNγ and effector cells positive for asialoGM1 and/or CD8. These results indicate that IL-15 is suitable for cancer immunotherapy in combination with IL-12.

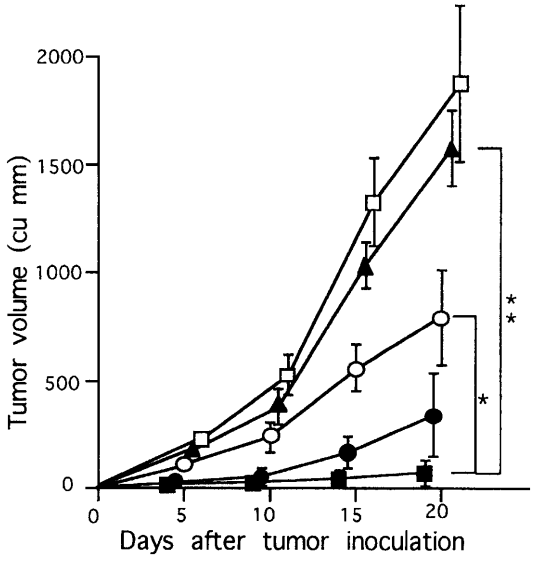

Fig. 5 Winn assay – effects of treatment with various Ab plus complement on the antitumor activity of non-adherent TEC of the rIL-15 + rIL-12-treated mice. MethA-bearing mice were injected with a combination of 100 ng rIL-15 and rIL-12 on days 0, 3, and 6. Non-adherent TEC were collected on day 7 and treated with various Ab plus complement in vitro. MethA (1×10^5 cells) were inoculated s.c. into normal BALB/c mice (seven mice per group) alone (□), or co-injected with 5×10^6 effector cells treated with complement alone (■), or with anti-CD8 mAb + complement (▲), or anti-CD4 mAb + complement (●), or anti-asialoGM1 mAb + complement (○). $^*P < 0.05$, $^{**}P < 0.01$ significantly different from the values for control mice co-injected with effector cells treated with complement only (by ANOVA)

IL-12 enhances the activity of NK cells [11], promotes development of Th1 cells and cytotoxic T cells [33] and enhances IFNγ production [21]. It has been shown to have potent antitumor and anti-metastatic activity in a number of tumor models including carcinoma, sarcoma, and melanoma [5, 8, 24]. It would thus appear that IL-12 is effective in antitumor resistance against malignant pleurisy. However, our study revealed that the antitumor activity of IL-12 alone was only marginal although it enhanced the ability of T cells in the TEC to produce IFNγ. On the other hand, IL-15 alone prolonged the survival time of mice inoculated i.pl. with MethA. IL-15, which uses the β and γ chains of IL-2R and the unique IL-15Rα for signal transduction [28], shares many of the biological activities of IL-2 including the ability to induce proliferation of NK cells, T cells and B cells [2, 3, 6, 13]. Besides biological activities shared with IL-2, IL-15 has unique activities; it activates macrophages to produce monokines including MCP-1 in the autocrine pathway [1] and has a chemotactic activity against macrophages, NK and T cells [7, 11, 20, 23, 25]. Furthermore, the memory phenotype of $CD8^+$ T cells and a significant fraction of intestinal intraepithelial lymphocytes expand preferentially in response to IL-15 [14, 34]. In the present results, the number of TEC increased drastically in mice treated with IL-15, whereas a similar increase in the number of TEC was not evident in mice treated with IL-12 alone following tumor inoculation. These results suggest that IL-15 may play an important role in provoking accumulation and expansion of inflammatory cells including NK cells, which may, in turn, provide antitumor activity against the malignant pleurisy.

IL-15 is less active in inducing of IFNγ production [21], while IL-12 is known to have a strong ability to induce IFNγ production by both resting and activated NK and T cells [22, 30]. Consistent with these findings, our results showed that IFNγ production by thoracic T cells was augmented by treatment with IL-12 but not by IL-15. Experiments with anti-IFNγ mAb reveal that IFNγ is important for protection by combination therapy with IL-15 and IL-12. IFNγ are cytokines that play an important role in host defense against various tumors through activation of phagocytes, stimulation of antigen-presenting cells, and differentiation of Th1 cells and cytotoxic T cells [4]. IFNγ also acts on tumor cells through increased sensitivity to apoptosis and inhibition of angiogenesis [4]. Such a variety of effects of IFNγ may be responsible for the protection against the malignant pleurisy.

Recently, IL-15 has been reported to up-regulate IL-12Rβ1 in resting PBMC, which are important for production of IFNγ and proliferation of NK and Th1 cells

[33]. In fact, IL-15 and IL-12 are shown to stimulate IFNγ production by NK and T cells [29] synergistically. We speculate that IL-15 provokes up-regulation of IL-12Rβ1 and consequently promotes activity of IL-12 against NK and T cells to induce IFNγ production. Taking into account all the data, it would thus appear that IL-15 and IL-12 may act synergistically to augment effector mechanisms via accumulation of effector cells and augmentation of IFNγ production by them. A large dose of IL-12 often provokes side-effects such as sudden death and hepatic failure [9]. Our results suggest that IL-15 may reduce the amount of IL-12 for cancer immunotherapy, which in turn prevents the side-effects.

IL-12 and IFNγ are thought to be important for induction of the Th1 response mediated by $CD4^+$ T cells [30]. Hence, IL-15 in synergy with IL-12 may also serve to induce a Th1 response in $CD4^+$ T cells against MethA. We have recently found that subcutaneous inoculation with MethA transfected with the IL-15 gene provoked strong antitumor immunity mediated mainly by MethA-specific $CD4^+$ T cells [15]. Our present results, however, reveal that protection by intrapleural administration with exogenous IL-15 and IL-12 is mainly dependent on asialoGM^+ cells and/or $CD8^+$ T cells but not on $CD4^+$ cells. The effector mechanisms against tumors in the pleural cavity may be different from those in subcutaneous tumors. We have previously reported that intrapleural administration of heat-killed cells of *Lactobacillus casei* YIT 9018 (LC9018) resulted in a strong antitumor activity in a murine malignant pleurisy model in which BALB/c mice were transplanted i.pl. with syngeneic MethA [17–19]. This antitumor effect was accompanied by increased cytotoxic activities of NK cells, and augmentation of several cytokines including IFNγ, TNFα and IL-1β. Depletion of effector cells by in vivo treatment with anti-CD3, CD4, CD8 mAb revealed that $CD8^+$ T cells were partly responsible for the antitumor effect of i.pl. administration of LC9018 [17]. In our preliminary study, we found that the macrophages in TEC from LC9018-treated mice expressed increased levels of IL-12 and IL-15 mRNA and that neutralizing endogenous IL-12 and IL-15 by in vivo administration of mAb against these cytokines significantly inhibited the anti-tumor activity of LC9018. Thus, the antitumor mechanisms induced by treatment with IL-15 together with IL-12 may also be those induced by some bacterial components, although other inflammatory cytokines such as TNFα and IL-1β may be involved in their antitumor activity.

In conclusion, IL-15 in combination with IL-12 exhibited strong host-mediated antitumor activity in a malignant pleurisy model using BALB/c mice and syngeneic MethA. NK cells and $CD8^+$ T cells contributed to the antitumor effect of IL-15 and IL-12 treatment. A combination of IL-15 and IL-12 may be useful clinically in the immunotherapy of at least some cases of malignant pleurisy.

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