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Combined IL-15/IL-15Rα Immunotherapy Maximizes IL-15 Activity In Vivo

Thomas A. Stoklasek, Kimberly S. Schluns, and Leo Lefrançois

IL-15 has substantial potential as an immunotherapeutic agent for augmenting immune responses. However, the activity of IL-15 is mediated by a unique mechanism in which the cytokine is transpresented by cell-bound high-affinity IL-15Rα to target cells expressing the IL-15Rβ and the common γ-chain. Thus, the efficacy of administered IL-15 alone may be limited by the availability of free IL-15Rα. We now show that administration of soluble IL-15/IL-15Rα complexes greatly enhanced IL-15 half-life and bioavailability in vivo. Treatment of mice with this complex, but not with IL-15 alone, resulted in robust proliferation of memory CD8 T cells, NK cells, and NK T cells. The activity of the complex required IL-15Rβ, but not IL-15Rα, expression by the responding cells and was IL-7-independent. Interestingly, IL-15/IL-15Rα immunotherapy also caused naive CD8 T cell activation and development into effector cells and long-term memory T cells. Lastly, complexed IL-15, as compared with IL-15 alone, dramatically reduced tumor burden in a model of B16 melanoma. These findings hold significant importance for the use of IL-15 as a potential adjuvant/therapeutic and inducer of homeostatic proliferation, without the necessity for prior immunodepletion. The Journal of Immunology, 2006, 177: 6072–6080.
have found that IL-15 therapy, in conjunction with chemotherapy, TLR agonists, or adoptive transfer of tumor-reactive CD8 T cells, results in increased survival or complete tumor regression in mouse tumor models, in contrast to each therapy alone (48–50). Thus, manipulation of IL-15 activity has potential as a therapeutic modality in a number of clinical situations.

Considering these findings and the transpresentation model, we hypothesized that IL-15 action in vivo could be augmented by the administration of soluble IL-15 (sIL)-15Ra and IL-15 complexes. Our results show that forced transpresentation of IL-15 in vivo profoundly enhanced IL-15 activity and drove proliferation and differentiation of IL-15-responsive immune cells. Importantly, complexes of IL-15, in contrast to IL-15 alone, reduced B16 tumor burden in a systemic tumor model. These findings hold important ramifications for the future of IL-15-targeted therapy.

Materials and Methods

Mice and cells
C57BL/6-Ly5.1 mice were purchased from The Jackson Laboratory. C57BL/6-Ly5.2 mice were purchased from Charles River Laboratories. The OT-I mouse line was provided by Dr. W. R. Heath (Walter and Eliza Hall Institute, Australia) and Dr. F. Carbone (University of Melbourne, Parkville, Australia) and was maintained as a C57BL/6-Ly5.2 line on a RAG-2−/− background. IL-15Ra−/− mice (8) were provided by Dr. A. Ma (University of California San Francisco, CA). Spleen cells from IL-2Rα−/− mice were provided by Dr. M. Farrar (University of Minnesota, Minneapolis, Minnesota). IL-7−/− mice (51) were originally obtained from DNAx Research Institute and were maintained on a C57BL/6 background. All procedures were conducted under National Institutes of Health guidelines and were approved by the institutional animal care committee. Memory CD8 T cells were generated by adoptive transfer of CD45.1 OT-I-RAG−/− cells to CD45.2 B6 mice that were then infected with vesicular stomatitis virus (VSV)-OVA. Alternatively, to produce VSV nucleoprotein-specific memory cells, CD45.1 B6 mice were infected i.v. with 1 × 10⁶ PFU VSV-Indiana. In either case, at least 60 days after infection, enriched CD8 T cells containing OT-1 or VSV-specific memory cells were used in adoptive transfer studies.

CFSE labeling of cells and adoptive transfer
Single-cell suspensions were created in HBSS by homogenizing spleens or lymph nodes using frosted glass slides. RBC were lysed, and splenocytes were filtered through Nitex. Cells were incubated for 10 min at 37°C with CFSE (2 μM; Molecular Probes), the reaction was quenched with HBSS with 5% FCS (52), and the cells were washed twice. CFSE-labeled cells were resuspended in PBS and injected i.v. into congenic mice.

IL-15 treatment
Human IL-15 was generously provided by Amgen. Recombinant mouse IL-15Ra-Fc chimeric molecule was purchased from R&D Systems. Human IL (hIL)-15 and recombinant murine (rm)IL-15Ra-Fc, both suspended in PBS, were mixed and incubated for 30 min at 37°C. Each mouse, unless specifically noted, received 2.5 μg of IL-15 either alone or precomplexed with 15 μg of rmIL-15Ra-Fc in 200 μL of PBS i.p.

Flow cytometric analysis
Cells were isolated at the indicated times and analyzed for the presence of donor cells using CD45 allele status and their expression of surface markers and CFSE intensity. The percentage of cells of the original population donor cells using CD45 allele status and their expression of surface markers and their expression of surface markers and their expression of surface markers.

ELISA for detection of IL-15
Anti-hIL-15 (MAB647; R&D Systems) in PBS (5 μg/ml, 100 μl/well) was added to 96-well high binding plates (3500; Corning) at 37°C for 1 h. The plates were washed and then blocked using PBS/1% BSA/0.2% Tween 20 (200 μl/well) for 1 h at 37°C. Dilutions of serum (in blocking buffer) were incubated for 1 h at 37°C, followed by washing with PBS/0.05% Tween 20 and the addition of biotinylated anti-hIL-15 Ab (ABM247; R&D Systems; 0.2 μg/ml, 100 μl/well) for 1 h at 37°C. Finally, Avidin-HRP (BD Pharmingen) (1/1000 dilution) was added for 1 h at 37°C. After washing, TMB substrate (34021; Pierce) was added, and 1 M phosphoric acid was used to stop the reaction. ODs at 450–570 nm were measured using a microplate reader (Bio-Rad; model 680). The serum half-life of IL-15 was calculated using the medical calculator provided by Cornell University per the given instructions (http://www-users.med.cornell.edu/~spong/picu/calculator.html).

In vivo cytotoxicity assay
This assay was performed essentially as described previously (56). Normal spleen cells were labeled to low (0.25 μm) or high (2.5 μm) CFSE levels, and CFSE+ cells were incubated with 1 μg/ml SIINFEKL peptide for 45 min at 37°C. Equal numbers (10⁴) of each population were mixed and injected i.v. into OT-I-transferred mice that were either untreated or treated with IL-15/IL-15Ra or were infected with 1 × 10⁵ PFU of VSV-expressing chicken OVA (57) 4 days earlier. Four hours later, spleen cells were analyzed for the presence of CFSElow and CFSE+ populations. Percentage of CFSElow and CFSE+ populations. Percentage of lysis = [1 − (ratio unprimed/ratio primed)] × 100. Ratio = percentage of CFSElow:percentage of CFSE+.

Intracellular detection of IFN-γ
Lymphocytes were isolated from the spleen and cultured for 5 h with 1 μg/ml Golgistop (BD Pharmingen), with or without 1 μg/ml of the OVA-derived peptide SIINFEKL. After culture, cells were stained for surface molecules then fixed, and cell membranes were permeabilized in Cytofix/ Cytoperm solution (BD Pharmingen) and stained with anti-IFN-γ PE or control rat IgG1 PE. Cells were then washed, and the fluorescence intensity was measured on a FACSCalibur (BD Biosciences).

Tumor challenge and treatment
B16-F1 cells were maintained in Advanced DMEM (Invitrogen Life Technologies) supplemented with 10% FCS/100 U/ml penicillin/100 μg/ml streptomycin/430 μg/ml Glut-Max. Cells were harvested using 0.25% trypsin/EDTA (Invitrogen Life Technologies) when 50–80% confluent, and 1 × 10⁶ cells (in PBS) were injected via the lateral tail vein. On day 1 and day 10 post-B16 injection, mice began receiving treatment i.p.: PBS, IL-15 (2.5 μg), or IL-15-R-Fc (2.5 μg/15 μg). Mice were sacrificed on day 21. For tumor examination, mice were randomized and scored in a blinded fashion.

Results

Coadministration of IL-15 and IL-15Ra drives CD8 memory T cell and NK cell proliferation in vivo
To determine whether precomplexed cytokine and soluble receptor would augment IL-15 activity in vivo, IL-15 and IL-15Ra-Fc (IL-15Ra) were incubated at a 1:1 molar ratio before injection. At this ratio, because rmIL-15Ra-Fc is dimeric, one binding site per rmIL-15Ra-Fc is theoretically filled. Increasing by 2-fold the amount of IL-15 added to the receptor did not increase activity (data not shown). Human and mouse IL-15 provoked similar responses in our model, although hIL-15 exhibited somewhat higher activity than mouse IL-15 (data not shown). To measure IL-15-mediated activity in vivo, we used an adoptive transfer model to gauge the effect of IL-15 on the proliferation of CD8 T cells. CD45.1 CFSE-labeled CD8-enriched splenocytes were transferred to normal CD45.2 mice that were then treated with PBS, IL-15 alone (2.5 μg), IL-15Ra (15 μg), or a mixture of IL-15 (2.5 μg) and IL-15Ra (15 μg). Four days after treatment with IL-15 alone, 8.4% of the donor CD8 T cell population had divided (Fig. 1A, top panels), in agreement with our previous results (23). In dramatic contrast, the coadministration of the same amount of IL-15 bound to IL-15Ra resulted in the proliferation of 64.3% of the donor CD8
T cells (Fig. 1A, top panels). Furthermore, whereas the majority of CD8 T cells responding to IL-15 alone divided once, the cells responding to combination treatment underwent 5–7 divisions, resulting in a substantial increase in donor cell numbers (data not shown). Importantly, administration of IL-15Rα alone did not induce proliferation of CD8 T cells (Fig. 1A, top panels). The bulk of the dividing cells expressed high levels of CD44, suggesting that the responding cells were primarily memory CD8 T cells or that CD44 had been up-regulated (Fig. 1A, bottom panels). To test the action of combined therapy on bona-fide memory CD8 T cells, we adoptively transferred CFSE-labeled nucleoprotein-specific memory CD8 T cells that had been generated by infection with VSV. Similar to the above results, Ag-specific memory CD8 T cells responding to combined IL-15/IL-15Rα treatment proliferated to a much greater extent than those provided IL-15 alone (Fig. 1B) and increased in number (data not shown).

Past studies have implicated IL-15 as an inducer of B cell, NK cell, and NK T cell proliferation (58–63). Therefore, we examined the ability of IL-15 and receptor-complexed IL-15 to induce proliferation of these cell types using the adoptive transfer system. CD4 T cells did not proliferate in response to 2.5 μg of IL-15 (Fig. 2). The responding CD4 T cells tended to express high levels of CD44 (data not shown). Of interest, the polyclonal CD8 T cell population, Ag-specific memory CD8 T cells, and NK cells also exhibited signs of activation 1 day posttreatment in terms of CD69 up-regulation and CD127 down-regulation (data not shown).

Coadministration of IL-15 and IL-15Rα-Fc enhances CD8 T cell proliferative response to exogenous IL-15. A, On day −1, mice received 1.5 × 10^7 congenic CFSE-labeled, CD8-enriched lymphocytes i.v. and were treated i.p. on day 0 with PBS, IL-15 (2.5 μg), IL-15Rα-Fc (15 μg), or IL-15Rα-Fc (15 μg) with IL-15 (2.5 μg). CD8^+ splenocytes were analyzed on day 4 by flow cytometry for CFSE fluorescence and CD45.1 expression (top panels), or CD45.1^+ CD8^+ cells were analyzed for CFSE fluorescence and CD44 expression (bottom panels) (n = 4). Data are representative of three similar experiments. B, On day −1, mice received CFSE-labeled CD8 T cell-enriched splenocytes containing ~6.5 × 10^5 tetramer^+ VSV nucleoprotein-specific memory CD8 T cells and were treated on day 0 with PBS, IL-15 (2.5 μg), or IL-15Rα-Fc (15 μg) with IL-15 (2.5 μg). Donor tetramer^+ splenocytes were analyzed by flow cytometry on day 4 for CFSE fluorescence. R = the percentage of responding cells.

Complexed IL-15/IL-15Rα greatly enhances IL-15 activity in vivo

We next examined the early kinetics of the CD8 T cell-proliferative response to the coadministration of IL-15Rα with IL-15. CFSE dilution was negligible 1 day after treatment, but by day 2 33% of the donor CD8 T cell population had divided (Fig. 3). By day 3, 66% of donor CD8 T cells had divided with many cells in divisions 5–6, whereas 74% had divided by day 4 with some cells in their 7th round of division. By day 20 after treatment, many of the cells had completely diluted their CFSE, although cells at intermediate stages of division remained. These results showed that the maximum effect of a single dose of IL-15/IL-15Rα was achieved by ~4 days posttreatment.

To obtain an approximation of the level of activity enhancement obtained by combined treatment over that of IL-15 alone, we performed titrations of IL-15 and IL-15/IL-15Rα using the adoptive transfer system. Comparisons were based on the extent of donor CD8 T cell proliferation as assessed by CFSE dilution. A dose of 0.1 μg of IL-15 combined with 0.6 μg of IL-15Rα induced a level of proliferation similar to that of 5 μg of IL-15 (Fig. 4A). Thus, in this type of experiment, IL-15 activity was enhanced ~50-fold by coadministration with IL-15Rα. Considering this substantial enhancement, we questioned whether IL-15 alone could achieve a similar level of activity. Even with the administration of 37.5 μg
of IL-15, the level of proliferation obtained with 0.5 μg of receptor-complexed IL-15 could not be achieved (Fig. 4B). When examining the NK and NK T cell response to 37.5 μg of IL-15, the proliferation induced was nowhere near the level achieved by 0.5 μg of IL-15 complexed with IL-15Rα (3.0 μg) (Fig. 4C). We also noted that the CD8 T cell proliferation induced by IL-15 alone plateaued at ~12 μg of cytokine and did not increase with increasing dosage (data not shown). These results suggested that IL-15 half-life and/or IL-15Rα availability were limiting in vivo.

**Complexing IL-15 to IL-15Rα greatly increases half-life and serum levels of IL-15**

The effect seen by complexing IL-15 and soluble receptor may operate in part by increasing the half-life and bioavailability of exogenously administered IL-15. To test this possibility, we developed an ELISA to detect hIL-15 in mouse serum. Preliminary experiments indicated that complexing hIL-15 with IL-15Rα did not interfere with Ab binding (data not shown). Mice were treated i.p. with hIL-15 (2.5 μg) alone or a mixture of hIL-15 and IL-15Rα (3 μg) and serum was obtained at various times after treatment, as well as just before treatment. We noted that the half-life of hIL-15 alone was ~1 h, whereas when complexed to the receptor, IL-15 half-life was extended to ~20 h (Fig. 5A). With regard to maximum serum levels obtained, IL-15 alone peaked at a concentration of ~70 ng/ml 30 min after administration, whereas complexed IL-15 peaked 2 h after administration at a concentration of ~600 ng/ml. Similar results were noted with administration via the i.v. route, indicating that the differences noted were not due to differences in absorption from the peritoneal cavity (data not shown). Using this assay, we also determined that the presence or absence of endogenous IL-15Rα did not affect the serum levels or kinetics of administered hIL-15 (data not shown), suggesting that IL-15 binding to endogenous IL-15Rα did not contribute significantly to the short half-life of IL-15. The serum levels of IL-15 when administered as a receptor complex also correlated with functional activity as measured by CFSE dilution of transferred cells (Fig. 5B). By 24 h after administration, activity had declined substantially and by day 4 after treatment, no activity was detected by this assay.

**Receptor-complexed IL-15 functions through IL-15Rβ**

The effects of complexed IL-15/IL-15Rα could either be mediated by direct or indirect effects on the responding cell types. If direct, then it might be expected that the target cells would be required to express IL-15Rβ component(s). To test this, we transferred CFSE-labeled CD45.1 IL-15Rα−/− CD8 T cells into CD45.2 IL-15Rα−/− hosts and treated the mice with either IL-15 or complexed IL-15/IL-15Rα. IL-15 alone could not be transpresented in the absence of endogenous IL-15Rα, and did not induce proliferation (Fig. 6A) (23). In contrast, donor CD8 T cells from IL-15/ rmIL-15Rα-treated mice proliferated extensively. Furthermore, the IL-15Rα−/− donor cells, which primarily consisted of naive phenotype CD8 T cells, progressively increased their expression of CD44 and CD122 with division (Fig. 6A).

Because responding T cells did not require IL-15Rα to respond to complexed IL-15/IL-15Rα, we examined the role of IL-15Rβ (CD122) in mediating this effect, the expression of which is required for transpresentation activity of IL-15 (23). To this end, we transferred CFSE-labeled CD45.2 CD122−/− or CD122−/− CD8 T cells into CD45.1 B6 mice and analyzed the donor cells for CFSE dilution 4 days after treatment. Although control cells proliferated vigorously in response to IL-15/IL-15Rα treatment, CD122−/− donor CD8 T cells did not proliferate in response to coadministration (Fig. 6B). Importantly, endogenous CD8 T cells in both groups expanded in response to treatment. Similar results were

**FIGURE 4.** Coadministration of IL-15Rα-Fc with IL-15 greatly enhances IL-15 potency. A. On day −1, mice received 1.5 × 10⁶ congenic CFSE-labeled, CD8-enriched lymphocytes i.v. and on day 0 received either PBS (data not shown), IL-15 (5 μg), or varying doses of IL-15/IL-15Rα-Fc (0.5 μg + 3 μg) i.p. (n = 3). Data are representative of two similar experiments. B and C. On day −1, each mouse received 4.5 × 10⁶ congenic CFSE-labeled, CD8-enriched lymphocytes i.v. and on day 0 received either PBS (data not shown), IL-15 (0.5 μg) + IL-15Rα-Fc (3 μg), or IL-15 (37.5 μg) i.p. CD8⁺ splenocytes were analyzed on day 4 for CFSE dilution by flow cytometry (n = 3). Data are representative of two similar experiments.

**FIGURE 5.** Complexing IL-15 with IL-15Rα-Fc increases half-life and bioavailability of exogenous IL-15 in the serum. A. Mice were injected with hIL-15 (2.5 μg) i.p. with or without precomplexed IL-15Rα-Fc (15 μg). Mice were bled over time (0.25, 0.5, 1, 2, 4, 7, 16, 21, 44, 79, and 110 h after treatment), and hIL-15 presence in mouse serum was monitored using a hIL-15-specific ELISA. Data are representative of two similar experiments with three mice per group. B. Mice were injected with either PBS on day 0 or IL-15 (2.5 μg) and IL-15Rα-Fc (15 μg) complex on day −4, day −1, or day 0 i.p. On day 0, all mice received 1 × 10⁶ CFSE-labeled CD45.1 CD8 T cells i.v. Splenocytes were examined on day 4 posttransfer for CFSE dilution. Experiment is representative of two similar experiments with three mice each.
obtained when CD122-blocking Ab was used to prevent IL-15 signaling (data not shown). Taken together, the results indicated that IL-15/IL-15Rα operated via direct transpresentation through interaction with the IL-15Rβ likely in conjunction with γC.

**Proliferation induced by forced IL-15 transpresentation does not require IL-7**

Because IL-7 is essential for the homeostatic proliferation of CD8 T cells in immunodeficient hosts (64), we wished to test the role of IL-7 in proliferation induced by receptor-bound IL-15. Congenic CFSE-labeled CD8 T cells were transferred to control or IL-7−/− mice, and combined IL-15/IL-15Rα was administered. The absence of IL-7 had no effect on CD8 T cell proliferation in response to IL-15 with IL-15Rα (Fig. 7), indicating that IL-7 was not involved in IL-15-mediated proliferation in our system.

**IL-15/IL-15Rα immunotherapy induces naive T cell activation and effector function**

In previous experiments, we noted that CD44low polyclonal CD8 T cells as well as naive TCR transgenic T cells responded to IL-15 when coadministered with IL-15Rα (Figs. 1 and 6). Considering that under homeostatic conditions CD8 memory T cells exhibit much greater responsiveness to IL-15 than do naive CD8 T cells, we wished to directly compare the responsiveness of these two subsets to complexed IL-15/IL-15Rα. To do so, CFSE-labeled memory OT-I and naive OT-I CD8 T cells were adoptively transferred into the same congenic C57BL/6 hosts, and proliferation was analyzed 4 days after treatment with IL-15/IL-15Rα. Surprisingly, naive OT-I CD8 T cells proliferated almost as well as memory OT-I CD8 T cells (Fig. 8A). The naive OT-I cells also expanded ~10-fold in response to the complex as compared with controls and up-regulated CD44 (Fig. 8B). Similar results were seen with FACS-purified CD44low OT-I cells (data not shown); thus, any contaminating CD44high naive OT-I cells cannot account for the naive CD8 T cell proliferation seen. We also examined the activation status of naive OT-I cells 1 day after treatment by measuring the expression level of CD69, which is up-regulated after T cell activation, and IL-7Rα (CD127), which is down-regulated after T cell activation (64). Interestingly, CD69 was increased and CD127 was decreased on OT-I cells 1 day after treatment with IL-15/IL-15Rα (Fig. 8C).

In light of the robust proliferation induced in naive T cells, it was of interest to establish whether effector function was concomitantly induced. To test this possibility, we adoptively transferred
naive OT-I CD8 T cells into congenic C57BL/6 hosts and, using an in vivo cytotoxicity assay, measured Ag-specific lytic activity 4 days after treatment with IL-15/rmIL-15Rα or after infection with recombinant VSV-expressing OVA (VSV-OVA) for comparison. Interestingly, IL-15/rmIL-15Rα treatment resulted in induction of robust Ag-specific lytic activity, similar to the level obtained with virus infection (Fig. 9A). In addition to lytic activity, the majority of naive OT-I CD8 T cells activated by IL-15/rIL-15Rα or VSV-OVA infection produced high levels of IFN-γ following in vitro restimulation with peptide (Fig. 9B). This result was in contrast to the negligible frequency of OT-I cells producing IFN-γ from control (PBS) and IL-15-treated mice (Fig. 9B). Thus, the induction of effector function in naive CD8 T cells by coadministration of IL-15Rα with IL-15 paralleled the activation obtained by infection.

**Treatment of naive T cells with complexed IL-15/IL-15Rα generates memory CD8 T cells**

Although naive T cells developed into effector cells in response to transplanted IL-15, it remained to be seen whether this was a transient effect or resulted in memory T cell development. Therefore, we analyzed the number and phenotype of OT-I cells 44 days after naive OT-I T cell transfer and IL-15/IL-15Rα treatment. At this time point, a ~5-fold higher percentage of OT-I cells was present following IL-15/IL-15Rα administration as compared with untreated mice, as well as greater OT-I numbers (Fig. 10, top panels; data not shown). Moreover, nearly all of these cells expressed high levels of CD44 and CD122 (Fig. 10, middle and bottom panels). We have also noted that memory OT-I cells induced by receptor-complexed IL-15 were able to produce IFN-γ upon peptide restimulation to a similar extent as bona-fide Ag-experienced OT-I cells (data not shown). Thus, in the absence of Ag, IL-15/IL-15Rα treatment was able to induce the development of memory CD8 T cells.

**FIGURE 9.** Receptor-complexed IL-15 induces effector function in naive CD8 T cells. A, On day −1, B6 mice received 2.5 × 10⁵ naive OT-I-RAG⁺⁺ cells and were treated with PBS, IL-15 (2.5 μg), IL-15Rα-Fc (15 μg) with IL-15 (2.5 μg) i.p., or 1 × 10⁶ PFU of VSV-OVA i.v. On day 4 posttreatment, each mouse received a mixture of CD45.1/2 CFSE-labeled (1.5 μM) nonpeptide-pulsed splenocytes and CFSE-labeled (0.0015 μM) SIINFEKL peptide-pulsed splenocytes. Four hours later, splenocytes were analyzed for the presence of the CFSE-labeled target populations (data shown) (n = 4). Data are representative of two experiments. Percentage shown is percentage of Ag-specific killing per group ± SD. B, On day −1 CD45.2 mice received ~2 × 10⁶ naive CD45.1 OT-I-RAG⁺⁺ cells and on day 0 were treated with PBS, IL-15 (2.5 μg), IL-15Rα-Fc (15 μg), and IL-15 (2.5 μg), or 1 × 10⁶ PFU VSV-OVA i.v. On day 4, splenocytes were incubated in vitro with or without SIINFEKL peptide for 5 h, and the production of IFN-γ OT-I (gated on CD45.1 donor cells) was analyzed by intracellular staining (n = 3). Data are representative of two similar experiments. Percentage shown is percentage of the gated OT-I donor population staining for intracellular IFN-γ.

**FIGURE 10.** IL-15/IL-15Rα-Fc treatment generates memory cells from naive CD8 T cells. On day −1, B6 mice received ~6 × 10⁶ CD45.1 CFSE-labeled naive OT-I-RAG⁺⁺ cells and on day 0 were treated i.p. with PBS or IL-15 (2.5 μg) and IL-15Rα-Fc (15 μg). Forty-four days later, splenocytes were analyzed for percentage of donor OT-I CD8 T cells (top panels) and OT-I expression of CD44 and CD122 (middle and bottom panels). Percentages shown are from individual mice (n = 2). Data are representative of two experiments.

**IL-15/IL-15Rα acts as an antitumor immunotherapeutic agent**

Because IL-15/IL-15Rα leads to the expansion and activation of CD8 T cells and NK cells, two populations known to be involved in tumor surveillance, we wished to compare the ability of IL-15 vs IL-15/IL-15Rα in enhancing tumor immunity. To this end, we injected 1 × 10⁵ B16-F, melanoma cells i.v. on day 0 and treated mice with either PBS, IL-15 (2.5 μg), or IL-15/IL-15Rα (2.5 μg/15 μg) i.p. on day 1 and day 10. This tumor protocol leads to the establishment of tumors in the lung and liver. Mice were examined and scored for the presence of tumor nodules. We found that 90% of the PBS and IL-15-treated mice (compiled from two separate experiments) were tumor positive (Fig. 11) and exhibited a similar tumor burden between groups (multiple tumors >5 mm in diameter), whereas only one of the IL-15/IL-15Rα-treated mice was tumor positive and contained only a single 2-mm lung tumor. These results indicated a potential therapeutic value of combining IL-15 and IL-15Rα to prevent tumor engraftment.
Discussion

Recent findings support the use of IL-15 as an adjuvant for vaccination, tumor immunotherapy, and immune system reconstitution in immunodeficiency (50, 65–67). In the case of cancer treatment, induction of lymphopenia is now being used to enhance the functional activity of adoptively transferred lymphocytes (68–72). This modality is based on the finding that CD8 T cells undergoing lymphopenia-driven homeostatic proliferation differentiate into effector cells with lytic and cytokine-producing activities (73, 74). Thus, the proliferation and functional activities induced by the IL-15/IL-15Rα complex in intact hosts mimicked homeostatic proliferation triggered by lymphopenia.

Noteworthy, the level of proliferation obtained by treatment with the complex could not be achieved by high doses of IL-15 alone. Because the same cell producing IL-15 may also transiently express the cytokine (23–25, 75), the availability of free IL-15Rα may be limited thus restricting the effectiveness of treating with IL-15 alone. The fact that the serum half-life or level of exogenous IL-15 was not significantly altered by the presence or absence of endogenous IL-15Rα (data not shown) further supports the theory that free IL-15Rα is limiting in vivo. Our results demonstrated that IL-15/IL-15Rα recapitulated IL-15 responsiveness in an IL-15Rα−/− host. This was illustrated by the proliferation of IL-15Rα−/− donor CD8 T cells in an IL-15Rα−/− host, as well as the induction of memory phenotype CD8 T cells and NK cells (data not shown), that are normally lacking in IL-15Rα−/− mice. This effect highlighted the potential of receptor-complexed IL-15 to reestablish the cytolytic arm of the immune system during states of lymphopenia. In addition, our studies showed that the short half-life of IL-15 was extended ~20-fold when complexed to the receptor. Equally impressive was the notable increase in the serum availability of IL-15 when administered bound to IL-15Rα. Indeed, when complex was delivered i.v., nearly all of the administered IL-15 was accounted for in the serum some 15 min later, whereas when uncomplexed, only 4% of the IL-15 dose could be detected in the serum (data not shown). Therefore, treatment with IL-15 alone is unlikely to achieve the full therapeutic potential of the cytokine. The combined administration of IL-15/IL-15Rα may provide improved efficacy by driving transpresentation through available IL-15RβγC.

The activity of complexed IL-15/IL-15Rα appears to be mediated at multiple levels. Mortier et al. (26) recently demonstrated that IL-15 bound to sIL-15Rα has a higher affinity for IL-15RβγC than does free IL-15. Thus, from our data and that of others, binding of IL-15 by IL-15Rα-chain can increase IL-15 potency by 1) increasing IL-15 half-life (Fig. 4), 2) increasing IL-15 affinity for IL-15RβγC (26), and 3) providing a platform for transpresentation. Future work will examine the mechanisms of the IL-15Rα-mediated increase in IL-15 half-life. Possible mechanisms involve 1) protection of IL-15 from degradation by proteases, 2) inhibition of clearance via receptor binding or other mechanisms, or 3) FcR-mediated binding/recycling of complex. In regard to the latter, although the presence of the Ig Fc portion in the complex could augment activity through FcR-mediated signaling, IL-15 bound to monomeric sIL-15Rα lacking a Fc, retained function in vivo, although a quantitative comparison with the Fc-containing molecule was not performed (data not shown).

The ability of IL-15/IL-15Rα to drive T cell activation was of particular interest given the current paradigm regarding the requirements for naive and memory T cell homeostatic survival and proliferation. Under normal conditions, survival of both naive and memory CD8 T cells requires IL-7 (64), whereas IL-15 is essential for homeostatic proliferation of memory CD8 T cells (9) and NK cell survival (24, 76) in normal hosts. In a lymphopenic environment, IL-7 is required for homeostatic proliferation of naive CD8 and CD4 T cells (64), and plays a role, along with IL-15, in mediating CD8 memory T cell homeostatic proliferation (62). Thus, it was unexpected that naive CD8 T cells responded vigorously to the IL-15/IL-15Rα complex. It should be noted, however, that in IL-15−/− mice, the naive CD8 T cell pool is decreased ~50%, suggesting that either naive CD8 T cell development and/or survival requires IL-15 (7). In any case, proliferation of naive, as well as memory phenotype, CD8 T cells driven by receptor-bound IL-15 was IL-7 independent and required IL-15Rβ signaling. This result indicated that naive CD8 T cells expressed sufficient levels of IL-15Rα to respond to IL-15/IL-15Rα but not to sIL-15 alone. Previous reports also showed that IL-15 activates naive human CD8 T cells in vitro (77, 78). Interestingly, the naive CD8 T cell response to cytokine paralleled that of an Ag-specific response, although the response was driven by unphysiological levels of IL-15 activity. Nevertheless, clonal expansion occurred, and effector function was induced, followed by contraction of the responding population and generation of memory CD8 T cells. Phenotypic changes similar to those observed following TCR triggering also accompanied activation via receptor-complexed IL-15, with CD69 levels up-regulated and IL-7Rγc down-regulated early after treatment. This process occurred in the absence of Ag, although whether MHC is necessary for these events is currently under investigation. A previous in vitro study showed that IL-15 induced a similar activation and genetic profile in human CD8 memory T cells as did TCR cross-linking (79), suggesting some overlap in the signaling pathways activated by these receptors. Additional experimentation will be needed to decipher the underlying mechanisms of naive CD8 T cell activation mediated by IL-15.

Our findings also highlight the potential of receptor-complexed IL-15 as a cancer therapeutic. Two doses of complexed IL-15 were able to lead to the rejection (or prevent the establishment) of B16 melanoma tumors, whereas IL-15 alone was not able to diminish tumor burden. Future studies will examine the effect of IL-15/IL-15Rα on more established tumors using a variety of doses and treatment schedules, as well as determining the mechanism and cell types involved in tumor rejection.

These findings present a conundrum, considering that sIL-15Rα has been used to inhibit collagen-induced arthritis (29), cardiac allograft rejection (30), delayed type hypersensitivity (75), and allergic airway disease (80). Interestingly, initiation of all of these conditions is dependent, either solely or in part, on CD4 T cells, suggesting that CD4 T cells may respond to IL-15 indirectly. Thus, IL-15 may activate dendritic cells during certain immune responses (75) leading to CD4 T cell activation, and this event could hypothetically occur via direct IL-15 action through IL-15Rα in the absence of IL-15Rβ, rather than through transpresentation. In this scenario, activation of CD4 T cells, and in some cases CD8 T cells, could be inhibited by administration of sIL-15Rα if levels of free IL-15 are elevated, as is known to occur in inflammatory diseases. Recent data (81) also show that a cell surface form of IL-15 exists whose function in vivo remains obscure, but binding of sIL-15Rα to such a molecule could also exert potential inhibitory effects. Some divergence of the response toward CD8 and NK/NK T cell activation through treatment with sIL-15Rα may also result in inhibition of certain immune responses. Further studies are needed to determine the parameters that determine whether inhibition or augmentation of immune responses is the outcome of manipulation of the IL-15 system.

During the course of our studies, we became aware of the work of Rubinstein et al. (82), whose study also shows that complexing sIL-15Rα to IL-15 results in hyperagonist activity toward CD8
11. Goldrath, A. W., P. V. Sivakumar, M. Glaccum, M. K. Kennedy, M. J. Bevan, M. J. Milliron, M. A. Ma, and A. Ma. 2001. Coordinate expression and transpresentation of interleukin (IL-15)−15Rα/15Rα and IL-15 induces robust NK/NK T and CD8 T cell expansion and effector differentiation in intact hosts, which may have important immunotherapeutic applications.

Disclosures

The authors have no financial conflict of interest.

References


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