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Converting IL-15 to a superagonist by binding to soluble IL-15Rα

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IL-15 is normally presented *in vivo* as a cell-associated cytokine bound to IL-15Rα. We show here that the biological activity of soluble IL-15 is much improved after interaction with recombinant soluble IL-15Rα; after injection, soluble IL-15/IL-15Rα complexes rapidly induce strong and selective expansion of memory-phenotype CD8$^+$ cells and natural killer cells. These findings imply that binding of IL-15Rα to IL-15 may create a conformational change that potentiates IL-15 recognition by the βγc receptor on T cells. The enhancing effect of IL-15Rα binding may explain why IL-15 normally functions as a cell-associated cytokine. Significantly, the results with IL-2, a soluble cytokine, are quite different; thus, IL-2 function is markedly inhibited by binding to soluble IL-2Rα.

cytokines | T cells | soluble receptors | CD122 | natural killer cells

In mice, certain cells, namely memory-phenotype (MP) CD8$^+$ T cells and natural killer (NK) cells, are highly sensitive to IL-15 (1–9). MP CD8$^+$ cells display high levels of CD44 and, like NK cells, also show high expression of CD122 (IL-2Rβ), a component of the receptor for both IL-15 and IL-2 (6). For resting cells, responsiveness to these two cytokines is controlled by a two-chain receptor, βγc, consisting of the β chain (CD122) plus the common γ chain, γc, which controls intracellular signaling.

IL-15 is normally not secreted in soluble form (8–10) but is held on the cell surface bound to a unique receptor, IL-15Rα, especially on dendritic cells (11–16). Cell-bound IL-15 then is presented in trans to T cells and NK cells and is recognized by the βγc receptor on these cells; such recognition maintains cell survival and intermittent proliferation.

IL-15Rα plays a mandatory role in presenting endogenous IL-15. Thus, like IL-15$^{-/-}$ mice (1), IL-15Rα$^{-/-}$ mice lack CD122^{hi} CD8$^+$ cells and NK cells (17), presumably because the IL-15 synthesized in IL-15Rα$^{-/-}$ mice fails to leave the cytoplasm. Nevertheless, βγc$^+$ cells can proliferate in response to a soluble recombinant form of IL-15 in the absence of IL-15Rα (18). Moreover, under certain conditions, IL-15Rα can be inhibitory. Thus, injecting mice with a soluble (s) recombinant form of IL-15Rα is reported to suppress NK cell proliferation (10) and certain T dependent immune responses *in vivo* (19–22), and adding sIL-15Rα *in vitro* can block the response of cell lines to IL-15 (20–25). Despite these findings, there are other reports that sIL-15Rα (26), and also a soluble sushi domain of IL-15Rα (27), can enhance IL-15 responses of human cell lines.

In this paper, we investigated whether sIL-15Rα can alter the response of normal mouse T cells to IL-15. As discussed below, IL-15 responses of CD8$^+$ T cells and NK cells are improved considerably by association with sIL-15Rα, both *in vitro* and *in vivo*.

Results

Stimulation by IL-15/IL-15Rα Complexes *in Vitro*. To examine whether the stimulatory function of soluble IL-15 is altered by binding to sIL-15Rα, purified MP CD44^{hi} CD122^{hi} CD8$^+$ cells were cultured *in vitro* with mouse IL-15 ± mouse sIL-15Rα covalently linked to the Fc portion of human IgG1 (sIL-15Rα-Fc). For IL-15 alone, half-maximal responses required ≈30 ng/ml and responses were negligible with <10 ng/ml (Fig. 1*A* and *B*). Here, the notable finding was that supplementing a low concentration of IL-15, e.g., 5 ng/ml, with sIL-15Rα-Fc led to strong proliferative responses of MP CD8$^+$ cells as measured either by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution (Fig. 1*A*) or by [^{3}H]thymidine incorporation (Fig. 1*B*). No proliferation occurred with sIL-15Rα-Fc alone (Fig. 1*B*), and the addition of sIL-15Rα-Fc failed to alter the response of MP CD8$^+$ cells to a different cytokine, IL-2 (data not shown). With IL-15, sIL-15Rα-Fc did not appear to act by enhancing the half-life of IL-15 *in vitro* (Fig. 6, which is published as supporting information on the PNAS web site).

With a limiting concentration of cytokine, IL-15 responses were improved generally by 6- to 9-fold by the addition of sIL-15Rα-Fc. Adding sIL-15Rα-Fc also considerably improved the IL-15 response of CD122^{hi} NK cells (Fig. 1*C*) but was relatively ineffective on MP (CD44^{hi}) CD4$^+$ cells, which express intermediate levels of CD122 (Fig. 1*C*). Unexpectedly, sIL-15Rα-Fc plus IL-15 led to significant proliferation of typical naïve CD44^{lo} CD122^{lo} CD8$^+$ cells, although only with high concentrations of IL-15 (Fig. 1*C*).

For MP CD8$^+$ cells, responses to both soluble IL-15 alone and IL-15 plus sIL-15Rα-Fc were mediated solely through βγc receptors. Thus, responses were abolished by addition of CD122 mAb (Fig. 1*D*) and were as high with MP CD8$^+$ cells from IL-15Rα$^{-/-}$ mice as with normal MP CD8$^+$ cells (Fig. 1*E*).

Being a dimeric molecule, sIL-15Rα-Fc might enhance IL-15 activity by presenting this cytokine in a cross-linked form. However, enzyme-cleaved monomeric fragments of sIL-15Rα (free of Fc) were no less potent in augmenting IL-15 responses than dimeric molecules (Fig. 1 *A* and *B*). Indeed, under limiting conditions, responses were appreciably higher with the receptor monomers than with the dimers (Fig. 1*B*). Why the receptor monomers were more effective than the dimers is unclear, although for steric reasons, the monomer/IL-15 complexes may bind more effectively to the βγc receptor.

The above data refer to mouse IL-15 and mouse soluble IL-15Rα. Quite similar data applied to human IL-15/IL-15Rα. Thus, the response of mouse MP CD8$^+$ cells to either human or mouse IL-15 was enhanced considerably by the addition of human sIL-15Rα-Fc (Fig. 7, which is published as supporting information on the PNAS web site). The addition of human IL-15Rα monomers was even more effective (data not shown).

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CFSE, carboxyfluorescein diacetate succinimidyl ester; MP, memory-phenotype; NK, natural killer; s, soluble.

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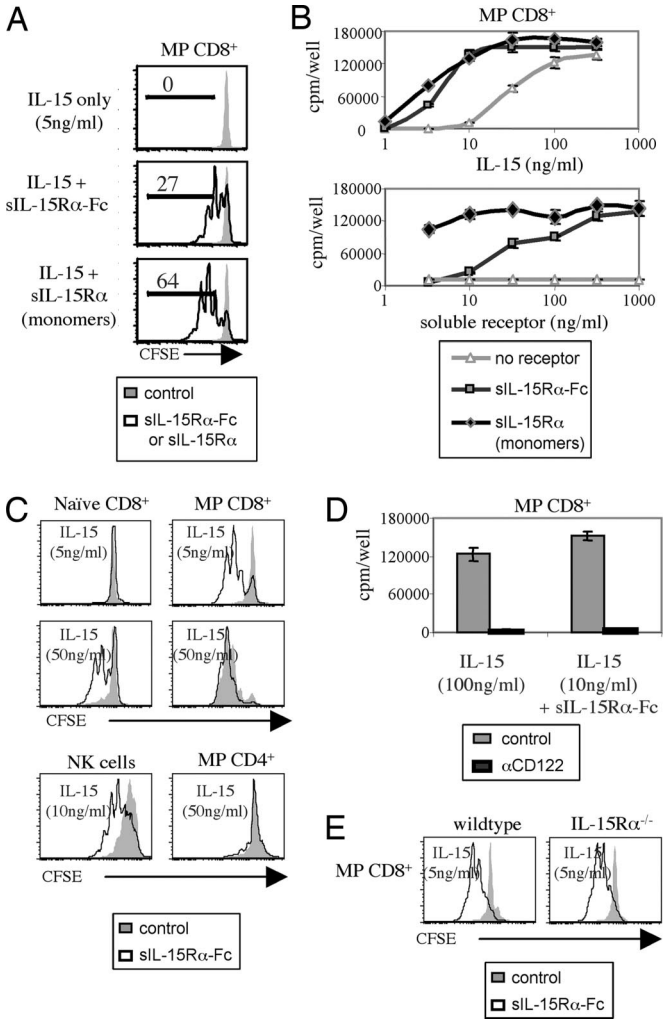

Fig. 1. Soluble IL-15Rα augments IL-15-mediated lymphocyte proliferation *in vitro*. (*A*) Purified MP ($CD44^{hi}$) $CD8^+$ T cells from IL-7 tg mice were labeled with CFSE and cultured at 5×10^4 cells per well with 5 ng/ml of IL-15. As indicated, 1 μg/ml of either sIL-15Rα-Fc (dimers) or sIL-15Rα (monomers) was added to the cultures. CFSE dilution was assessed on day 4. Representative data are shown. (*B*) Purified MP $CD8^+$ T cells were cultured with either titrated amounts of IL-15 plus a fixed concentration of soluble receptor (1 μg/ml) (*Upper*) or titrated amounts of soluble receptor plus a fixed concentration of IL-15 (10 ng/ml) (*Lower*). The data show mean levels of $[^3H]$thymidine incorporation (±SD) for triplicate cultures on day 3. (*C*) Purified naïve ($CD44^{lo}$) $CD8^+$ T cells, MP $CD8^+$ T cells, NK cells, or MP $CD4^+$ T cells were cultured with IL-15 as indicated. Soluble IL-15Rα-Fc was added at 1 μg/ml. CFSE dilution was assessed on day 3. (*D*) Same as in *B* except 10 μg/ml of anti-CD122 antibody was added as indicated. (*E*) MP $CD8^+$ T cells from wild-type IL-7 tg (Ly5.2) and IL-$15R\alpha^{-/-}$/IL-7 tg (Ly5.1) mice were mixed together, labeled with CFSE, and cultured as indicated. CFSE dilution on $Ly5.1^-$ (wild type) and $Ly5.1^+$ (IL-$15R\alpha^{-/-}$) cells was measured on day 3.

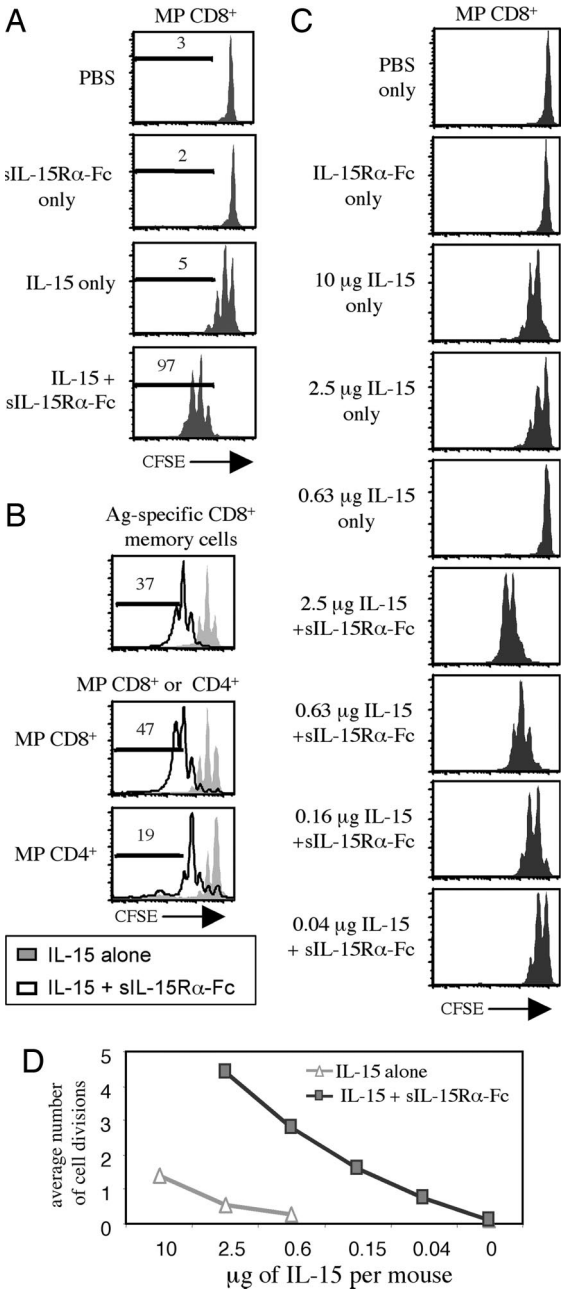

Fig. 2. Soluble IL-15Rα augments IL-15-mediated donor lymphocyte proliferation *in vivo*. (*A*) CFSE-labeled T cells were transferred i.v. into C57BL/6 (B6) recipients. On days 1 and 2 after transfer, the recipients were given i.p. injections of PBS, sIL-15Rα-Fc alone (7 μg), IL-15 alone (1.5 μg), or sIL-15Rα-Fc plus IL-15 (7 μg and 1.5 μg, respectively, which represents a 1:2 molar ratio). CFSE dilution of the donor cells was measured in spleen on day 4. Representative data for gated MP $CD8^+$ cells are shown. (*B*) As in *A* except that the cells transferred were from lymphocytic choriomeningitis virus-immune mice (*Upper*) versus normal mice (*Lower*). (*C*) CFSE-labeled MP $CD8^+$ T cells were transferred to normal B6 hosts; one day later, the hosts were injected with the indicated dose of IL-15 with or without sIL-15Rα-Fc; the dose of sIL-15Rα-Fc varied such that a 2:1 molar ratio of IL-15 to sIL-15Rα-Fc was injected. CFSE profiles for donor MP $CD8^+$ cells in spleen at 2 days after injection are shown. (*D*) Compilation of data from *C*. For *A*–*C*, data shown are representative of two mice per group and are also representative of two independent experiments.

Note that for $\beta\gamma_c$ responses, human IL-15 is considerably weaker than mouse IL-15 (25).

For the *in vivo* experiments discussed below, we used mouse sIL-15Rα-Fc. Insufficient amounts of the monomers were available for *in vivo* studies.

***In Vivo* Responses.** Confirming previous findings (2, 5), injecting mice i.p. with IL-15 after i.v. injection of CFSE-labeled MP $CD8^+$ cells caused ≈50% of the donor cells to divide one to two times (Fig. 2*A*). With coinjection of sIL-15Rα-Fc, however, virtually all of the donor cells divided and >95% of the cells divided three times or more (compared with <5% for IL-15 injected alone); by contrast, injection of sIL-15Rα-Fc alone had no effect on proliferation. The capacity of sIL-15Rα-Fc to

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enhance responses of MP CD8$^+$ cells to IL-15 also applied to antigen-specific memory CD8$^+$ cells, i.e., to antigen-primed P14 TCR Tg CD8$^+$ cells (Fig. 2*B Upper*). There was also enhancement of the IL-15 response of MP CD4$^+$ cells (Fig. 2*B Lower*).

For MP CD8$^+$ cells, IL-15 titration experiments showed that *in vivo* responses to IL-15 were increased ≈50-fold when limiting doses of IL-15 were coinjected with sIL-15Rα-Fc (Fig. 2 *C* and *D*). Part of this increase in biological activity appears to reflect enhanced *in vivo* lifespan of IL-15 when bound to sIL-15Rα-Fc, because IL-15/sIL-15Rα-Fc complexes displayed prolonged biological activity compared with free IL-15 (Fig. 8, which is published as supporting information on the PNAS web site).

The above data apply to CFSE-labeled donor cells. For host cells, injection of IL-15 or sIL-15Rα-Fc alone had little effect on cell numbers. By contrast, two injections of IL-15 plus sIL-15Rα-Fc caused a marked increase in total numbers of host MP CD8$^+$ cells and NK cells by day 3 after initial injection, and the spleen was obviously enlarged (Fig. 3 *A* and *B*). Likewise, proliferation as measured by BrdU incorporation was much higher with injection of IL-15 plus sIL-15Rα-Fc than with IL-15 alone (Fig. 3*C*).

IL-15Rα$^{-/-}$ mice lack CD122^{hi} MP CD8$^+$ cells and NK cells (17), presumably because the absence of IL-15Rα precludes presentation of endogenous IL-15. As shown in Fig. 3*D*, injecting IL-15Rα$^{-/-}$ mice with a mixture of IL-15 and sIL-15Rα-Fc rapidly restored numbers of host NK1.1$^+$ DX5$^+$ NK cells and CD122^{hi} MP CD8$^+$ cells; at the dose used, IL-15 alone was ineffective.

Failure of sIL-15Rα-Fc to Block Presentation of Endogenous IL-15. Injecting mice with LPS is known to cause a brief increase in endogenous IL-15 (and IL-15Rα) synthesis by non-T cells *in vivo*, with a consequent transient increase in the proliferation rate of IL-15-responsive CD122^{hi} MP CD8$^+$ cells (28, 29). Such LPS-induced bystander proliferation is illustrated in Fig. 4*A*, where most donor MP CD8$^+$ cells underwent one to two cell divisions by day 3 after exposure to LPS in normal B6 hosts, which contrasted with the lack of proliferation in IL-15Rα$^{-/-}$ hosts. Significantly, injecting sIL-15Rα-Fc after LPS injection failed to reduce proliferation, even with daily injections of sIL-15Rα-Fc (10 μg per injection). Hence, injection of sIL-15Rα-Fc was unable to block T cell contact with endogenous IL-15 bound to endogenous IL-15Rα. Also, for IL-15Rα$^{-/-}$ hosts, sIL-15Rα-Fc was clearly unable to compensate for the lack of endogenous IL-15Rα, presumably because the latter is essential for conveying IL-15 to the cell surface.

Similar findings applied to an *in vitro* system where MP CD8$^+$ cells were cultured in wells that were first coated with sIL-15Rα-Fc and then pulsed with IL-15, followed by thorough washing to remove unbound cytokine (Fig. 4*B*). Thus, proliferative responses elicited by the bound IL-15Rα-Fc/IL-15 complexes could not be inhibited by the addition of soluble (unbound) IL-15Rα-Fc as a blocking reagent. By contrast, addition of a polyclonal antibody against IL-15 abolished proliferation.

Based on the above findings, the IL-15 molecule has only a single binding site for interaction with IL-15Rα. Once this site is occluded, by binding either to endogenous IL-15Rα on cells *in vivo* or to IL-15Rα attached to plastic *in vitro*, interaction with exogenous sIL-15Rα-Fc fails to occur, and there is no interference with presentation of IL-15 to T cells. This scenario does not explain why sIL-15Rα can block the response of cell lines to IL-15 (20–25). Here it may be relevant that these studies used human or simian IL-15, and not mouse IL-15 as in our study, which raises the possibility of distinct species differences in IL-15. In favor of this idea, we found that, as for MP CD8$^+$ cells, the response of mouse CTLL cells to mouse IL-15 was enhanced by mouse sIL-15Rα-Fc (Fig. 9*A*, which is published as supporting information on the PNAS web site). By contrast, confirming the findings of others (20–23), the high response of CTLL cells to human IL-15 (25) was strongly inhibited by mouse sIL-15Rα-Fc

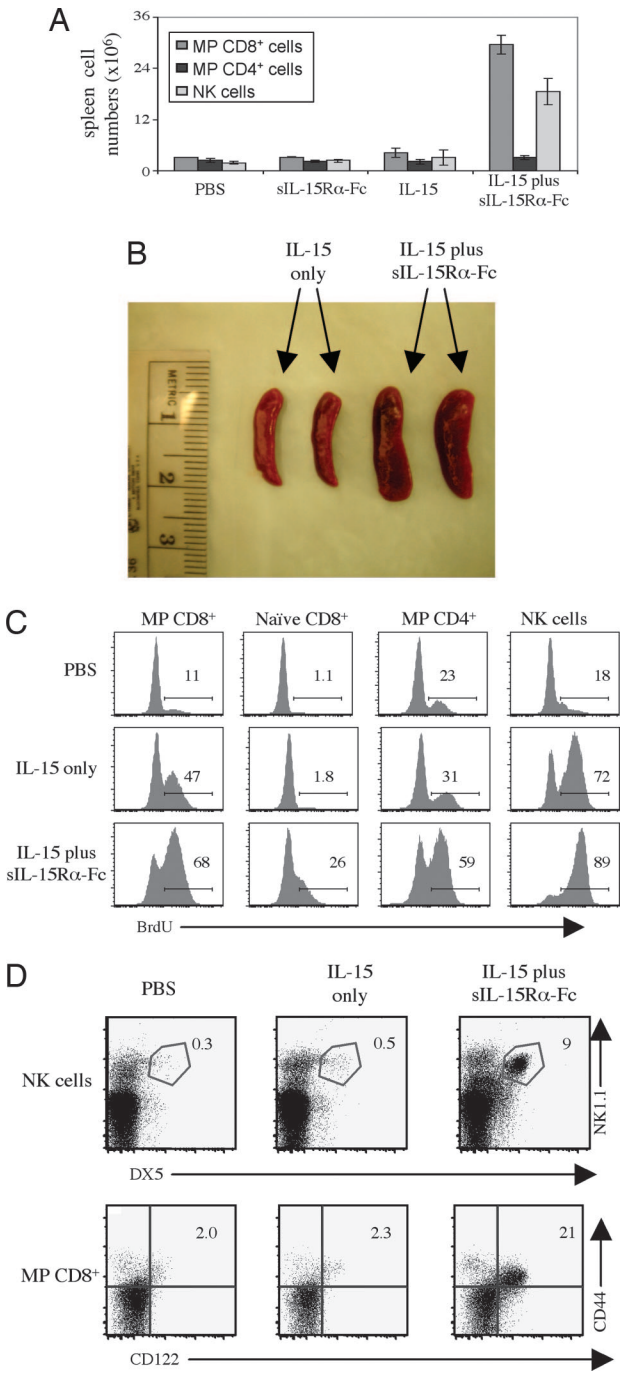
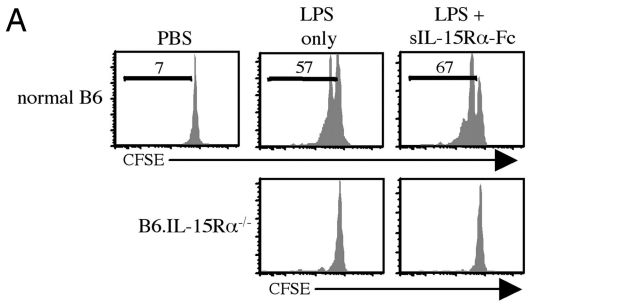
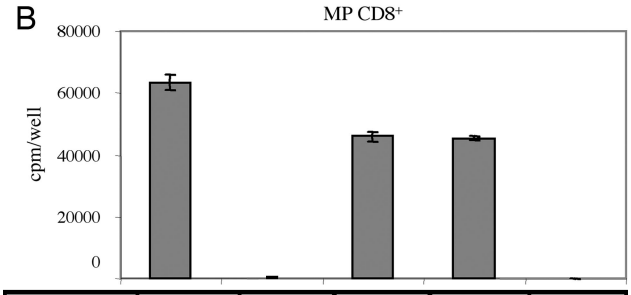


Fig. 3. Soluble IL-15Rα augments IL-15-mediated host lymphocyte proliferation. (*A*) Normal B6 mice were injected i.v. on days 1 and 2 with PBS, sIL-15Rα-Fc alone, IL-15 alone, or sIL-15Rα-Fc/IL-15 as described for Fig. 2*A*. Total numbers of CD8$^+$ MP T cells, CD4$^+$ MP T cells, and NK cells recovered from spleen on day 3 are shown. (*B*) Spleens from *A* were photographed as indicated. (*C*) Mice were treated as in *A*, except that the mice also were given an i.v. injection of BrdU on day 1 and placed on BrdU in the drinking water until killing. Shown is BrdU staining for MP CD8$^+$, naïve CD8$^+$, MP CD4$^+$, and NK cells. (*D*) IL-15Rα$^{-/-}$ mice were injected i.v. on days 1, 3, 5, and 7, with PBS, IL-15 (0.6 μg), or IL-15 (0.6 μg)/sIL-15Rα-Fc (3 μg). The data show staining of spleen cells on day 9. For *B–D*, representative data are shown. All data are representative of at least two independent experiments.





coating reagent	none	control	IL-15-Rα-Fc	IL-15-Rα-Fc	IL-15-Rα-Fc
pulse/wash	none	IL-15	IL-15	IL-15	IL-15
blocking reagent	none	none	none	IL-15-Rα-Fc	anti-IL-15
other	soluble IL-15	none	none	none	none

Fig. 4. Proliferation to IL-15 immobilized by IL-15Rα cannot be blocked by soluble IL-15Rα-Fc. (*A*) CFSE-labeled T cells were injected i.v. into Thy1-congenic B6 or IL-15Rα$^{-/-}$ hosts. One day later, mice were injected i.p. with PBS or 500 ng of LPS. As indicated, mice were also treated i.p. with 10 μg of sIL-15Rα-Fc daily beginning the day of LPS injection. Three days after LPS injection, spleens were harvested, and CFSE dilution of MP CD8$^+$ T cells was assessed. (*B*) Ninety-six-well plates were precoated overnight with 10 μg/ml of sIL-15Rα-Fc. Plates were then washed and incubated with 1 μg/ml IL-15 for 1 h at 37°C. Thereafter, plates were washed, and 5 × 10^4 MP CD8$^+$ T cells were added together with (*i*) 10 μg/ml sIL-15Rα-Fc, (*ii*) 10 μg/ml of polyclonal anti-IL-15 antibody, or (*iii*) control media; as an additional control, free IL-15 (32 ng/ml) was added to some wells. The data show mean levels of [^{3}H]thymidine incorporation (SD) for triplicate cultures on day 3.

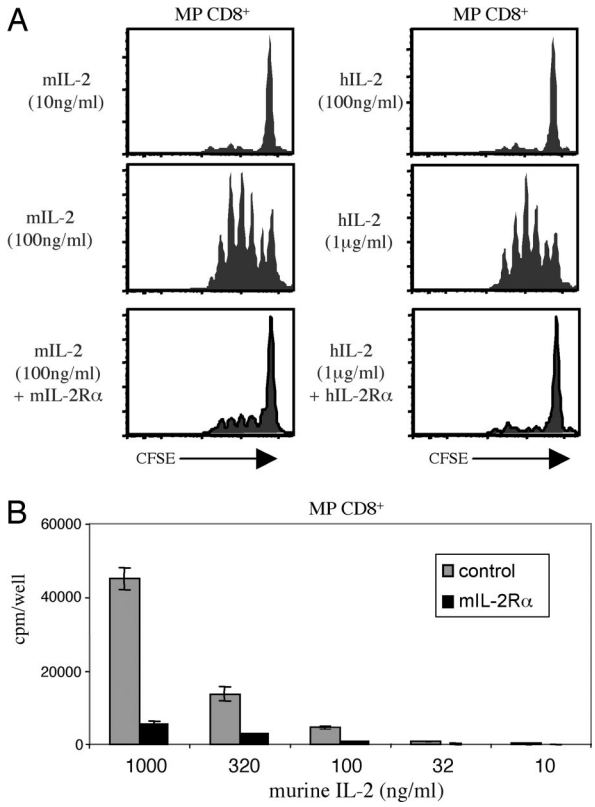


Fig. 5. Soluble IL-2Rα inhibits IL-2-mediated proliferation. (*A*) Purified CFSE-labeled MP CD8$^+$ T cells were cultured with either murine IL-2 or human IL-2 at the concentration shown. As indicated, 2.5 μg/ml of either soluble murine IL-2Rα or soluble human IL-2Rα was added to the cultures. CFSE dilution was assessed on day 3. Representative data are shown. (*B*) Purified MP CD8$^+$ T cells were cultured with titrated amounts of murine IL-2 with or without soluble mIL-2Rα (2.5 μg/ml). The data show mean levels of [^{3}H]thymidine incorporation (±SD) for triplicate cultures on day 3.

(Fig. 9*B*). CTLL responses to IL-2 as a control were not affected by adding sIL-15Rα-Fc (Fig. 9*C*).

The above findings do not explain the reports that murine sIL-15Rα constructs are inhibitory for NK cell proliferation (10) and antigen-driven T cell responses *in vivo* (19–22). This discrepancy has yet to be resolved, although it is of interest that antigen-specific proliferative responses of naïve OT-1 TCR Tg CD8$^+$ cells to specific peptide *in vivo* were not blocked by coinjection of sIL-15Rα-Fc, and the responses were considerably enhanced when a mixture of sIL-15R and IL-15 was injected (Fig. 9*D*). As yet we have not used the very large doses of sIL-15Rα required to block *in vivo* responses, i.e., 400 μg per injection for NK cell proliferation (10). Also, it is possibly relevant that the studies showing inhibition by sIL-15Rα *in vivo* used constructs grown in bacteria, whereas our constructs were grown in mammalian cells.

Stimulation by IL-2 Plus IL-2Rα. The observation that the biological activity of IL-15 was enhanced by binding to soluble IL-15Rα raised the question whether comparable findings would apply to IL-2 and IL-2Rα (CD25). As shown in Fig. 5, this possibility was clearly not the case. Thus, proliferative responses of MP CD8$^+$ cells to mouse IL-2 *in vitro* were inhibited markedly by the addition of soluble mouse IL-2Rα (Fig. 5*A Left* and *B*). Similar inhibition applied to MP CD8$^+$ cells (mouse) responding to human IL-2 and soluble human IL-2Rα (Fig. 5*A Right*).

Thus, whereas soluble IL-15Rα potentiated the function of IL-15, soluble IL-2Rα blocked the function of IL-2.

Discussion

The main conclusion from the above experiments is that soluble complexes of IL-15 and IL-15Rα are much more stimulatory than soluble IL-15 alone, both *in vivo* and *in vitro*. Without structural studies on IL-15/IL-15Rα interaction, one can only speculate on why and how this interaction potentiates IL-15 function. There are several possibilities.

First, binding of IL-15Rα to IL-15 might impair IL-15 internalization by T cells and, thereby, strengthen signaling through the βγ$_c$ receptor. This idea is in line with reports that internalization of certain cytokines, e.g., IL-2, serves to attenuate receptor signaling (30). However, we do not favor this possibility for two reasons. First, if the strong stimulation by IL-15/sIL-15Rα complexes reflected reduced IL-15 internalization, one would expect to see a parallel reduction in internalization of CD122, the receptor for IL-15. As measured by down-regulation from the cell surface, however, the opposite applies, i.e., greater down-regulation of CD122 with IL-15/sIL-15Rα complexes than with IL-15 alone (data not shown). The second argument against IL-15/sIL-15Rα complexes preventing IL-15 internalization is

that, if this possibility were the case, we should have seen similar findings with IL-2/sIL-2Rα, which was not so. Thus, IL-2/sIL-2Rα complexes were much less stimulatory than soluble IL-2 alone, which clearly contrasted with IL-15/sIL-15Rα complexes being more stimulatory than IL-15 alone.

A second possibility for how sIL-15Rα potentiates IL-15 activity is that sIL-15Rα might prevent degradation of IL-15. This notion deserves consideration because the enhancing effect of sIL-15Rα-Fc on IL-15 function was more pronounced *in vivo* than *in vitro*. Here, it is notable that binding of certain cytokines to antibodies or soluble receptors can extend cytokine survival *in vivo* (31–36). Consistent with this view, binding to sIL-15Rα-Fc did extend the half-life of IL-15 *in vivo*. However, additional mechanisms appear to be involved because sIL-15Rα-Fc improved the biological activity of IL-15 *in vitro* without affecting the cytokine half-life.

In light of the above findings, a third possibility needs to be considered, namely that IL-15Rα improves the function of IL-15 by inducing a conformational change in IL-15: this change augments interaction with the $\beta\gamma_c$ receptor, thus changing IL-15 from an agonist to a superagonist. This model is in line with the affinity of IL-15/IL-15Rα interaction being far higher than for IL-2/IL-2Rα interaction (3, 8) and explains why, unlike IL-2, IL-15 functions so well as a cell-associated cytokine. Testing this idea directly will obviously require structural studies. In this respect, it is notable that the interaction between IL-15 and IL-15Rα involves a unique network of ionic interactions not found in other cytokine/cytokine receptor complexes (37). Whether this unique interaction results in a conformational change in IL-15 has yet to be determined.

There is accumulating evidence that IL-15 has beneficial effects on T cell survival and memory generation and also has potential for restoring the T cell pool after irradiation and other forms of cytoreduction (4–9, 18, 38, 39). As shown here, the biological activity of IL-15 as a therapeutic reagent could be considerably enhanced by administering preformed soluble IL-15/IL-15Rα complexes.

Materials and Methods

Mice. C57BL/6 (B6), B6.Ly5.1, B6.Thy1.1, and OT-1 mice were purchased from The Jackson Laboratory. IL-15Rα$^{-/-}$ mice (17) were a generous gift of Averil Ma (University of California, San Francisco), and IL-7 transgenic (tg) mice (40) were a generous gift of J. Andersson (Basel Institute, Basel, Switzerland). P14 TCR tg mice were kindly provided by J. Lindsay Whitton (The Scripps Research Institute). IL-15Rα$^{-/-}$, IL-7 tg, P14, and OT-1 TCR tg mice were all maintained on a B6 background and, for some experiments, crossed to either B6.Ly5.1 or B6.Thy1.1 mice. IL-15Rα$^{-/-}$ mice were crossed to IL-7 tg mice to generate IL-7 tg/IL-15Rα$^{-/-}$ mice. As we have previously described with IL-7 tg/IL-15$^{-/-}$ mice (41), IL-7 tg/IL-15Rα$^{-/-}$ mice have similar large numbers of CD122^{hi} MP CD8$^+$ T cells as IL-7 tg mice.

Recombinant Proteins. Murine sIL-15Rα-Fc, human sIL-15Rα-Fc, and human IL-2Rα were purchased from R & D Systems. Monomeric sIL-15Rα and mouse IL-2Rα were purchased from R & D Systems as prerelease reagents. Monomeric sIL-15Rα was generated by the manufacturer by enzyme digestion of the dimeric sIL-15Rα-Fc, which resulted in the release of the Fc region. We verified complete digestion by Western blot with anti-IL-15Rα polyclonal antibodies (AF551, BAF551, and BAF847, R & D Systems) (data not shown). Recombinant cytokines (including mouse IL-15, human IL-15, mouse IL-2, human IL-2, mouse IL-4, and mouse GM-CSF) were purchased from eBioscience (San Diego) and/or R & D Systems.

Isolation of T Cells and CFSE Labeling. To obtain adequate numbers of cells, in most experiments, MP CD8$^+$ cells were prepared from IL-7 tg mice. By all parameters tested, MP CD8$^+$ cells from IL-7 tg mice are identical to cells from normal mice. Moreover, the main findings reported here for IL-15/sIL-15Rα-Fc complexes also were observed with cells prepared from normal mice, both *in vivo* and *in vitro*. MP CD8$^+$ T cells used for either *in vitro* or adoptive transfer experiments were isolated from lymph node (LN) and spleen and purified by cell sorting. In brief, single-cell suspensions were enriched first for CD3$^+$ T cells by using a mouse T cell enrichment columns (MTCC-25, R & D Systems). Enriched T cells were labeled with antibodies and purified by cell sorting for CD8$^+$CD44^{hi} T cells. In some experiments, we used a similar protocol and isolated CD8$^+$CD44^{lo}, CD4$^+$CD44^{hi}, NK1.1$^+$/DX5$^+$ cells. Cell sorting was performed by using a BD FACSAria (BD Biosciences). Purity of sorted cells was routinely tested and >98%. In some experiments, total T cells or OT-1 cells were used as donor lymphocytes. For these experiments, cells from spleen and LN were purified by using a mouse T cell enrichment column (MTCC-25). For experiments using CFSE-labeled cells, T cells were labeled with 1.5 μm CFSE (Molecular Probes) according to the manufacturer's directions.

Generation of Antigen-Specific CD8$^+$ T Cells. Lymphocytic choriomeningitis virus-specific P14 TCR tg CD8$^+$ T cells (Thy1.2/Ly5.2) were adoptively transferred into IL-7 tg recipient mice (Thy1.1/Ly5.2) and infected with 2×10^5 plaque-forming units of lymphocytic choriomeningitis virus Armstrong strain. Two months later, T cells were purified by using a mouse T cell enrichment column (MTCC-25), labeled with CFSE, and adoptively transferred into B6.Ly5.1-recipient mice (Thy1.2/Ly5.1). P14 CD8$^+$ T cells, which represented 15–20% of the donor CD8+ T cell population, were identified by triple staining for Thy1.2, Ly5.2, and CD8.

***In Vitro* Assays.** All cultures were performed in RPMI medium 1640 supplemented with 10% FCS, glutamine, 2-mercaptoethanol, nonessential amino acids, and antibiotics. FACS-purified T cells and NK cells were isolated as described above. CTLL (CTLL-2) cells were obtained from American Type Culture Collection (Manassas, VA), and cultured in RPMI medium 1640 supplemented with murine IL-2. For experiments with FACS-purified lymphocytes, 5×10^4 cells in 200 μl were plated per well in 96-well plates. Cytokine and/or soluble receptor were added at concentrations described in the figures. For CD122 blocking experiments, we used purified anti-CD122 antibody [TM-β1 (NA/LE); BD Pharmingen]. For experiments to block plate-bound IL-15, polyclonal anti-IL-15 antibody (AF447; R & D Systems) was used. Experiments with CTLL cells were plated as with FACS-purified lymphocytes except using 2×10^4 cells per well. For proliferation experiments with [^{3}H]thymidine, 1 μCi/ml (1 Ci = 37 GBq) was added as indicated in the figure legends. Cells were cultured in triplicate wells.

***In Vivo* Assays.** For experiments assessing proliferation of adoptively transferred cells, T cells were isolated and labeled with CFSE (as described above), and then injected i.v. into Ly5 or Thy1 congenic-recipient mice. In experiments to measure proliferation of host cells, mice were injected i.p. with BrdU (2 mg) and then maintained on BrdU drinking water (0.8 mg/ml) by using methodology described in ref. 2. For injections of cytokine and soluble receptor, IL-15 and sIL-15Rα-Fc were incubated together for 20 min at 37°C. Samples were then diluted at least 10-fold in PBS to a volume of 500 μl before injection into mice. In control conditions, cytokine or receptor alone also was incubated for 20 min at 37°C. LPS (ALX-581–008; Alexis Biochemicals, San Diego) were injected i.p. in PBS. For vaccination experiments, dendritic cells were prepared as described by culture of bone marrow cells with GM-CSF and IL-4 (38).

Dendritic cells were pulsed for 2 h with SIINFEKL peptide at 37°C, washed, and injected i.v.

Flow Cytometric Analysis. Cells were analyzed by flow cytometric analysis by using standard protocols. Briefly, cells were washed in FACS buffer containing 1% FCS and 2 mM EDTA and stained with combinations of the antibodies: CD8-PerCP-Cy5.5, -APC, or -APC-Cy7 (53-6.7; eBioscience and BD Pharmingen); CD49b-PE and -APC (DX5; eBioscience); NK1.1-FITC and -PE (PK136; BD Pharmingen); CD3-PE, -PerCP-Cy5.5, -PE-Cy7, or -APC (145–2C11; eBioscience and BD Pharmingen); CD3-Pacific Blue (500A2; BD Pharmingen); CD4-PE, PE-Cy7, or -APC (RM4-5, eBioscience and BD Pharmingen); Ly5.1-FITC, -PE, -PE-Cy7, and -APC (A20, eBioscience and BD Pharmingen); Ly5.2-FITC, -PE, -PerCP-Cy5.5, and -APC (104; eBioscience and BD Pharmingen); Thy1.1-FITC, PE, -PE-Cy7, and -APC (HIS51; eBioscience); Thy1.2-FITC, PE, and -APC (53–2.1; eBioscience); CD44-FITC-APC and -Alexa Fluor 405 (IM7, eBioscience and Caltag, Burlingame, CA); CD122-PE (TM-β1; BD Pharmingen); B220-PerCP-Cy5.5 (RA3–6B2; BD Pharmingen); and TCR Vα2-PE (B20.1, BD Pharmingen). BrdU intracellular staining was performed with reagents from FITC or APC BrdU flow kits (559619 and 552598; BD Pharmingen) according to the manufacturer's directions. Flow cytometric samples were analyzed by using a BD LSR II digital flow cytometer (BD Biosciences). Data were analyzed by using FLOWJO (Tree Star, San Carlos, CA).

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