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No Requirement of Trans Presentations of IL-15 for Human CD8 T Cell Proliferation

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The trans presentation of IL-15 by cells expressing the specific high-affinity receptor α-chain (IL-15Rα) to cells expressing the signaling receptor β-chain and γ-chain is essential for the generation and maintenance of CD8 memory T cells, NK cells, and NKT cells in an in vivo mouse system. We have also demonstrated in vitro that cell-surface IL-15Rα on cells expressing all the receptor components present IL-15 to receptor β-chain/γ-chain coexpressed on the same cell surface (cis presentation). However, although mouse CD8 T cells express all the IL-15R components, they show no evidence of cis presentation. In this study, we demonstrate that increased expression of mouse IL-15Rα in mouse CD8 T cells by retrovirus-mediated gene transfer changes the ability of the T cell to use cis presentation on the cell surface, indicating that cis presentation requires high expression of mouse IL-15Rα on the cell surface. Using cell lines expressing human or mouse receptors, we demonstrate that cis presentation occurs more efficiently in the human receptor–ligand combination than in that of the mouse system. Moreover, we found that primary human CD8 T cells do not require trans presentation of human IL-15 in vitro. These findings raise the possibility that the maintenance and generation of memory CD8 T cells are achieved via distinct mechanisms in humans and mice. Therefore, careful study of the human immune system, rather than extrapolation from the murine model, is necessary to achieve more complete understanding of memory CD8 T cell development in humans. The Journal of Immunology, 2010, 185: 6041–6048.

Interleukin-15 was initially identified as a cytokine capable of stimulating an IL-2–dependent cell line in vitro (1–3). IL-15 shares its overall structure with the cytokines of four α-helix bundles and also shares its signaling receptors CD122 (β-chain, referred to as Rβ) and CD132 (γ-chain, referred to as Ry) with IL-2 (2–4). A receptor chain specific to IL-15 (i.e., IL-15Rα) was later cloned based on its high-affinity binding to IL-15 (5). After these initial studies, the establishment of mouse lines lacking either IL-15 or IL-15Rγ clearly demonstrated the important roles of IL-15 in the development and maintenance of CD8 memory T cells, NK cells, and NKT cells in vivo (6, 7).

IL-15 binds to IL-15Rα with high affinity but with relatively low affinity to Rβ and Ry, which are responsible for signaling (2–5). Unlike the IL-2R system, in which IL-2Rα/β/γ binds co-ordinately to IL-2 to create a high-affinity receptor–ligand interaction for its physiological functions (8), there is no evidence of the coordinate binding of IL-15 to the IL-15Rα/β/γ expressed on the cell surface. Because mice lacking IL-15Rα are deficient in CD8 memory T cells and NK cells, membrane-bound IL-15Rα was originally considered to be relevant to IL-15 signaling on those cells (6, 9). However, it has since been clearly shown in a mouse system that IL-15Rα expression on hematopoietic cells is not essential for the maintenance of CD8 memory T cells or NK cells in vivo in the presence of IL-15Rα–expressing non-hematopoietic cells (10–12). It has been shown that IL-15Rα is expressed on a variety of cells and that cells expressing IL-15Rα can capture IL-15 and present IL-15 to T, NK, and NKT cells expressing Rβ/γ (13, 14). Further in vivo analysis demonstrated the importance of IL-15 production by non-hematopoietic cells expressing IL-15Rα for the maintenance of CD8 memory T cells and NKT cells (15). Based on these findings, together with the lack of evidence for soluble free IL-15 in vivo, it is proposed that IL-15 only exists as a complex with IL-15Rα on cells that express IL-15 and IL-15Rα or as a complex with soluble IL-15Rα that is generated by alternative splicing or proteolytic cleavage (16). Cells carrying the IL-15–IL-15Rα complex interact with T, NK, and NKT cells expressing Rβ/γ (trans presentation of IL-15). This interaction, but not the interaction between soluble IL-15 and IL-15Rα/β/γ expressed on cells, is necessary for the biological function of IL-15 in the mouse system.

These observations raise an intriguing question about the role of IL-15Rα expressed on T cells. In an in vitro single-cell assay using cells transfected with IL-15Rα (either the α-chain or β-chain alone or both the α-chain and β-chain together), we have previously demonstrated that cell-surface IL-15Rα can capture IL-15 and that this complex can interact with Rβ/γ on the same cell surface, exhibiting a highly sensitive response to IL-15 (cis interaction) (17). In this study, we demonstrate the mechanisms that regulate the trans and cis presentation of IL-15 by IL-15Rα to the Rβ/γ signaling receptor. We also show that mouse CD8 T cells, but not human CD8 T cells, are dependent on the trans presentation of IL-15 for their in vitro proliferation, suggesting a critical difference in the generation and maintenance of CD8 memory T cells in mice and humans.

Materials and Methods

Mice

IL-15Rα–deficient mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME), and the transgenic mouse line carrying the...
male Ag-specific I-restricted TCR genes (H-Y TCR mice) was a kind gift from Dr. Harald von Boehmer (Harvard Medical School, Boston, MA). B6 mice were purchased from Charles River Japan (Yokohama, Japan). All mice were maintained in the RIKEN Research Center for Allergy and Immunology animal facility under specific pathogen-free conditions.

**Cells and reagents**

The mouse mast cell line FDC3, expressing the endogenous Ry chain, was cloned from FDC-P1 cells purchased from the American Type Culture Collection (Manassas, VA) and was cultured in DMEM containing 10% calf serum and mouse IL-3 (mIL-3), cis-acting conditioned medium from an IL-3–expressing P815 cell culture. CTL-2 was purchased from American Type Culture Collection, and CTLL-JS is kindly provided by Dr. Mark Rubinstein and Dr. Jonathan Sprent (The Scripps Research Institute, La Jolla, CA). Both CTLJ sublines were cultured in DMEM containing 10% calf serum and human IL-2 (hIL-2)–containing conditioned medium from a hIL-2–expressing P815 cell culture. The Plat-E packaging cell line was obtained from Dr. Kitamura (Tokyo University, Tokyo, Japan) and cultured in DMEM containing 10% calf serum. Anti-mouse IL-15Rα (BASF51) and anti-mouse CD122 (R6, 13–1211) biotin-conjugated Abs were purchased from R&D Systems (Minneapolis, MN) and eBiosciences (San Diego, CA), respectively. PE-conjugated streptavidin and PE-conjugated anti-human CD4, anti-human CD8, and anti-mouse CD8 Abs were from BD Biosciences (San Jose, CA). Mouse mIL-15Rα (hIL-15Rα–Fc) were purchased from R&D Systems. Soluble mIL-15Rα was expressed and purified with a previously published protocol (18). Briefly, the cDNA encoding the full-length ectodomain of mIL-15Rα was amplified by PCR and subcloned into the pQE30 bacterial expression vector to express His-tagged soluble mIL-15Rα. The protein was expressed in *Escherichia coli* strain M15 cells and was purified to homogeneity by nickel-affinity and gel-filtration chromatography. To generate the complex, mIL-15–mIL-15Rα, mIL-15Rα and mIL-15 were expressed, refolded, and purified as described above and previously published protocol (17). Briefly, the DNA fragment encoding the full-length ectodomain of mature mIL-15Rα was amplified by PCR with primers 5′-CACCGGCTCTCCGGAGGACACACGGTGTCACC–3′ (forward) and 5′-GGGCGGGATCAATCAATCATTATCAAGAAGTGTTG-3′ (reverse). The DNA fragment encoding full-length mature mIL-15Rα was amplified by PCR with primers 5′-CACCGGCTCTCCGGAGGACACATGTGATCCTTATTGTT–3′ (forward) and 5′-GGGCGGGATCAATCAATCATTATCAAGAAGTGTTGATGAAATT-3′ (reverse). These DNA fragments were further amplified by PCR to add the T7 promoter, a sequence encoding a histidine affinity tag within the 5′ region, and the T7 terminator sequence within the 3′ region (19). These subclones were ligated into the TA vector PCR2.1TOPO (In- vitrogen, Carlsbad, CA) to express fusion proteins containing an N-terminal histidine tag and a tobacco etch virus protease cleavage site. Each of the proteins was expressed as a precipitate using the large-scale dialysis mode histidine tag and a tobacco etch virus protease cleavage site. For the infection of FDC3 cells expressing mIL-15Rα, in the presence or absence of soluble IL-15Rα (1 μg/ml). It should be noted that both monomeric and dimeric soluble mIL-15Rα proteins had identical biological functions in the proliferation assay in vitro. In the experiment to test the effect of cellular IL-15Rα (Supplemental Fig. 1), 10,000 cells/well of each cell type were cultured in round-bottom 96-well plates containing serial dilutions of recombinant IL-15. After incubation for 24 h, the cultures were pulsed with [3H]thymidine (1 μCi/well; GE Healthcare, Amersham, Buckinghamshire, UK) for 6 h and harvested on glass fiber filters for counting. To analyze the reactivity of CD8 T cells to IL-15, we cultured murine CD8 T cells activated with Con A (5 μg/ml), human CD8 T cells activated with PHA P (10 μg/ml), and an H-2-specific CD8 T cell line activated with H-2 Ag (all cell lines at 10,000 cells/well) in the manner described above for 3 d. The cultures were pulsed with [3H]thymidine (1 μCi/well) for 6 h and harvested on glass fiber filters for counting.

**Flow cytometric analysis**

The cells were washed with staining buffer (PBS containing 2% FCS and 0.02% sodium azide) and incubated with the indicated Abs for 15 min on ice. The samples were washed and further incubated with the appropriate secondary Ab for 15 min. The stained samples were analyzed on a FACSCalibur flow cytometer (BD Pharmingen) using the CellQuest software (BD Pharmingen) and FlowJo software (Tree Star, Ashland, OR).

**Results**

No cis presentation of IL-15 by IL-15Rα to Rb on activated murine CD8 T cells

We introduced either the mRβ gene alone or both the mIL-15Rα and mRβ genes into the FDC3 cell line, which expresses endogenous mRγ, and demonstrated the presence of both cis and trans presentation of mIL-15 (17). As shown in Supplemental Fig. 1, FDC3 cells expressing only mRβ responded to mIL-15, whereas FDC3 cells expressing only mIL-15Rα did not. In the presence of FDC3 cells expressing mIL-15Rα, the response of FDC3 cells expressing mRβ to mIL-15 was greatly enhanced, indicating that cellular mIL-15Rα enhances the reactivity to mIL-15 of cells expressing mRβ, similar to the results of a previous study (14). Previous studies by us and others have also shown that the effect of cellular IL-15Rα can be mimicked by soluble IL-15Rα protein (17, 21–23). As shown in Fig. 1A, cells expressing only mRβ responded poorly to mIL-15, but the addition of soluble mIL-15Rα to culture greatly enhanced the reactivity of the cells to mIL-15. The preformed complex of soluble mIL-15Rα–mIL-15 was also tested in this system. As shown in Supplemental Fig. 2, the complex stimulated the cells expressing mRβ as did mIL-15Rα together with soluble mIL-15Rα, indicating that the enhancing effect of mIL-15Rα is attributable to the formation of the complex, which has higher biological activity than mIL-15 alone. Thus, soluble mIL-15Rα, as well as cells expressing mIL-15Rα, enhanced the response to mIL-15 of cells expressing mRβ alone, indicating that the mIL-15Rα–mIL-15 complex, in either the soluble or cell-bound form, has higher biological activity on cells expressing mRβ than does mIL-15 alone.

The proliferative response of the FDC3 cell line expressing both mIL-15Rα and mRβ was more sensitive to mIL-15 than that...
of cells expressing mRb alone, and this response was inhibited by the addition of soluble mIL-15Rα (Fig. 1A). This strong sensitivity of cells expressing mIL-15Rα and mRb to mIL-15 did not require cell–cell contact and was mediated by the *cis* presentation of mIL-15 by cell-surface mIL-15Rα, as demonstrated previously (17). The inhibition induced by soluble mIL-15Rα is attributable to the competition between soluble mIL-15Rα and cell-bound mIL-15Rα for mIL-15. As Dubois et al. showed previously (14), cells expressing mIL-15Rα alone failed to respond to mIL-15. However, similar to an addition of soluble IL-15Rα protein, these cells also inhibited the response of the cells expressing both mIL-15Rα and mRb to mIL-15. It should be noted that in the presence of soluble mIL-15Rα, the proliferation curve of the FDC3 cells expressing mIL-15Rα and mRb or mRb alone almost overlapped, suggesting that under these conditions, the majority of mIL-15 molecules form complexes with the excess soluble mIL-15Rα to interact with the mRb/γ expressed on the FDC3 cells but do not interact with membrane-bound mIL-15Rα on the FDC cells expressing both receptors. These results indicate that cell-bound mIL-15Rα can present mIL-15 to mRb/γ on the same cell surface (*cis* presentation) more efficiently than can soluble mIL-15Rα.

When normal T cells were analyzed in the same in vitro assay, activated murine CD8 T cells responded poorly to mIL-15, whereas the addition of soluble mIL-15Rα enhanced their reactivity (Fig. 1B, left panel). Similar enhancement by mIL-15Rα was observed under the same conditions when naive CD8 T cells were assayed (Supplemental Fig. 3). This response was identical to that of CD8 T cells from IL-15Rα–deficient mice (Fig. 1B, right panel). However, the T cells from wild-type mice express both mIL-15Rα and mRb (Fig. 1C). These results indicate that the surface mIL-
15Rα expressed on the normal CD8 T cells does not contribute to the mIL-15 response of the cells in vitro.

Expression level of mIL-15Rα determines the trans or cis interaction in the mIL-15 response of murine T cells

The difference in the reactivity to mIL-15 of these two cell types (i.e., FDC3 cells expressing transfected IL-15 receptors and freshly activated CD8 T cells expressing endogenous IL-15 receptors) raises a question concerning the mechanisms regulating the cis and trans presentation of IL-15. Studies with an IL-2–dependent murine cell line, CTLL-2, provided a mechanistic explanation for this phenomenon. It has been shown that IL-15 reactivity is very heterogeneous among CTLL-2–derived variants. One of the cell lines studied by Rubinstein et al. (23) (CTLL-JS) showed very poor reactivity to IL-15 and a dependence on the trans presentation of mIL-15 by mIL-15Rα to enhance its reactivity. We tested two CTLL-2 sublines, one used in our laboratory for an IL-2 bioassay (CTLL) and the other from Rubinstein et al. (CTLL-JS). The CTLL cells exhibited very high sensitivity to mIL-15 in a proliferation assay, and the addition of soluble mIL-15Rα inhibited this response, indicating the presence of cis presentation (Fig. 2A, left panel). In contrast, the CTLL-JS cells showed very poor reactivity to mIL-15, and the addition of the soluble mIL-15Rα enhanced this response (Fig. 2B, left panel). The cDNAs of IL-15Rα expressed by both sublines were cloned and the sequences were shown to be identical (data not shown). Both cell lines express mRβ at similar levels (Fig. 2A, 2B, right panels). However, CTLL cells express significantly higher levels of mIL-15Rα than do CTLL-JS cells (Fig. 2A, 2B, right panels). Using retrovirus-mediated gene transfer, the mIL-15Rα cDNA under the control of a retroviral promoter was introduced into the CTLL-JS line, and cells expressing high mIL-15Rα were sorted (Fig. 2C, right panel). These cells exhibited higher sensitivity to mIL-15 than did the wild-type CTLL-JS cells, and their reactivity was inhibited by the addition of soluble mIL-15Rα (Fig. 2C, left panel). These results indicate that the surface expression level of mIL-15Rα is the factor that determines whether the cells respond to mIL-15 by cis or trans presentation. High levels of mIL-15Rα

![FIGURE 2. Response to IL-15 of CTLL sublines and cells transfected with mIL-15Rα cDNA. CTLL (A), CTLL-JS (B), and CTLL-JS (C) cell lines transfected with mIL-15Rα cDNA were tested for mIL-15 reactivity in the presence (solid circles) or absence (open circles) of soluble mIL-15Rα (1 μg/ml). The surface expression of mIL-15Rα and mRβ was determined as described in Fig. 1. The panels show the representative results of more than three experiments.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.064044)
on the cell surface seem to be required for the cis presentation of mIL-15 to the mRβγ on the same cell surface. In contrast, cells expressing low levels of mIL-15Rα still require trans presentation for enhanced sensitivity to mIL-15.

This phenomenon is not limited to the CTLL cell lines. The H-Y–Ag-specific CD8 T cell line was transfected with the sequence encoding mIL-15Rα and sorted for high surface expression of mIL-15Rα. The untransfected cells responded poorly to IL-15, and the addition of soluble mIL-15Rα enhanced their sensitivity to mIL-15 (Fig. 4A, 3B, left panel). In contrast, the cell line transfected with the sequence encoding mIL-15Rα showed higher sensitivity to mIL-15 in the proliferation assay, and this response was inhibited by soluble mIL-15Rα (Fig. 3A, 3B, right panels). These results indicate that normal CD8 T cells can also use cis-presented mIL-15 when they express high levels of mIL-15Rα.

Presence of cis and trans presentation of hIL-15 by hIL-15Rα

FDC3 cell line was transfected with sequences encoding hIL-15Rα and hRβ, or hRβ alone, and was tested for its reactivity to hIL-15. Like the cell lines expressing mouse receptors, the cells expressing hRβ showed low sensitivity to hIL-15, and their reactivity was enhanced by soluble hIL-15Rα (Fig. 4A, left panel). Cells expressing hIL-15Rα and hRβ responded to hIL-15 with exquisite sensitivity, and this response was inhibited by the addition of soluble hIL-15Rα (Fig. 4B, left panel). It should be noted that the sensitivity of FDC3 cells expressing human receptors to hIL-15 in a proliferation assay was significantly higher than that of the same cells expressing mouse receptors to mIL-15 (Fig. 4A, 4B, right panels). Nevertheless, the overall phenomenon was common to the human and mouse systems; that is, the high-level surface expression of IL-15Rα induced cis presentation and soluble IL-15Rα inhibited the effect of cis presentation, whereas soluble IL-15Rα enhanced the reactivity by trans presentation when there was no surface expression of IL-15Rα.

Although we attempted to examine the expression levels of hIL-15Rα by flow cytometry with several commercially available Abs, none of them showed a sufficiently intense signal for our purpose. Therefore, receptor expression was monitored by the expression of GFP, which was expressed as a bicistronic retroviral construct with m/hIL-15Rα. Because the IL-15Rα gene was transcribed together with the GFP gene as a single RNA, the fluorescence intensity of GFP should theoretically correlate with the expression level of IL-15Rα. m/hRβ expression was monitored similarly by the expression of human CD4, which was bicistronically expressed with m/hRβ. As shown in Fig. 4C, the expression of GFP on FDC3hαβ cells (Fig. 4C, left panel) was lower than the expression of GFP on FDC3αβ cells (Fig. 4C, right panel), suggesting that the expression of hIL-15Rα on FDC3αβ cells was lower than that of mIL-15Rα on FDC3αβ cells. Therefore, it seems that the expression level of hIL-15Rα does not explain why hIL-15 can be presented in cis more efficiently than can mIL-15. In other words, in the human system, cis presentation can even occur when IL-15Rα is expressed at low levels.

We also tested all combinations of mouse and human IL-15, as well as mouse and human soluble IL-15Rα, in a system with cells expressing either mIL-15Rα/mRβ, mRβ, hIL-15Rα/hRβ, or hRβ. The results are summarized in a table of EC50 values for each combination (Supplemental Table I). The proliferation of cells expressing mIL-15Rα and mRβ was induced by a small amount of hIL-15 (EC50 = 0.007 ng/ml) compared with the amount of mIL-15 required. This high activity was blocked by the addition of either soluble mIL-15Rα or hIL-15Rα (EC50 = 3 to 3.5 ng/ml). Conversely, neither soluble mIL-15Rα nor hIL-15Rα enhanced the activity of hIL-15 on cells expressing mRβ (EC50 = 4 to 5 ng/ml).

**FIGURE 3.** Effect of increased expression of mIL-15Rα on mIL-15 reactivity of the murine CD8 T cell line. A, H-Y–specific CD8 T cell line and the same line expressing transfected mIL-15Rα cDNA were tested for their response to mIL-15 in the presence (solid circles) or absence (open circles) of soluble mIL-15Rα (1 µg/ml), and the proliferative response was measured as described in Materials and Methods. B, Surface expression of mIL-15Rα and mRβ on the cell line used in A was determined by the methods described in Fig. 1. The panels show the representative results of more than three experiments.
ng/ml). Therefore, it seems that the enhancement of the biological activity of IL-15 by IL-15Rα is species specific; that is, soluble mIL-15Rα enhances the activity of mIL-15 on mouse cells, whereas soluble hIL-15Rα enhances the activity of hIL-15 on human cells. Mouse IL-15 had no effect on cells expressing either hIL-15Rα/hRβ or hRβ.

No evidence of trans presentation of hIL-15 to human activated CD8 T cells

Because the FDC3 assay showed that hIL-15 does not require highly expressed hIL-15Rα on the cell surface for its cis presentation, we wondered if human CD8 T cells react to hIL-15 by cis presentation. We compared the responses of activated human and murine CD8 T cells to IL-15 in vitro. As shown in Fig. 5A, ConA-activated murine CD8 T cells responded poorly to mIL-15, whereas soluble mIL-15Rα significantly enhanced their reactivity, indicating that murine CD8 T cells mainly react to the trans presentation of mIL-15. In contrast, PHA-activated human CD8 T cells showed higher sensitivity to hIL-15 than was observed in the murine system (Fig. 5B). The addition of soluble hIL-15Rα neither enhanced nor inhibited their response. These results indicate that, in contrast with murine CD8 T cells, which require the trans presentation of murine IL-15 to induce a highly sensitive “physiological” response to mIL-15, there is no evidence that human CD8 T cells require trans presentation of hIL-15.

Discussion

It is well established, at least in the murine system, that the presentation of IL-15 by cells expressing IL-15Rα to cells expressing Rβ/γ, such as CD8 T, NKT, and NK cells, is the dominant mode of interaction (trans presentation of IL-15). This trans presentation of IL-15, but not the direct response of the cells to soluble IL-15, is required for the generation and maintenance of CD8 T cells, NKT cells, and NK cells in vivo. Previous studies (14, 17, 21–23) and the results reported in this study demonstrate that IL-15Rα–bound IL-15 is more active than IL-15 alone on cells expressing Rβ/γ and that this higher biological activity of the IL-15–IL-15Rα complex plays an important role in the trans presentation of IL-15. The functional difference between IL-15 and the IL-15–IL-15Rα complex on cells expressing Rβ/γ is probably attributable to the structural differences between free IL-15 and

![FIGURE 4. Cis and trans presentation of hIL-15.](http://www.jimmunol.org/)

A, An FDC cell line expressing hRβ or mRβ was cultured with human (triangles) or mouse (circles) IL-15 in the presence (solid symbols) or absence (open symbols) of soluble human (triangles) or mouse (circles) IL-15Rα. Proliferation was measured as described in Materials and Methods. B, FDC cell lines expressing human (triangles) or murine (circles) IL-15Rα and Rβ were cultured as described in A, and their proliferation was measured as described in Materials and Methods. C, FDC cell lines expressing human Rαβ (hoβ; left panel) and murine Rαβ (moβ; right panel) receptors were stained with anti-human CD4 Ab as described in Materials and Methods. The cells were analyzed for the expression of GFP (bold line; correlates with IL-15Rα expression) and human CD4 (solid line; correlates with Rβ expression). Control staining of an untransfected FDC3 cell line (dotted line) is also shown. The panels show the representative results of more than three experiments.
IL-15 bound to the IL-15Rα. We and others have resolved the structure of the IL-15-IL-15R complex (17, 24), and we are currently analyzing the structure of free IL-15 to confirm this proposition.

In a previous report, we showed in a single-cell assay that cells expressing mIL-15Rα/β/γ respond to low doses of mIL-15 (17) and that this response is inhibited by the addition of soluble mIL-15Rα to the culture. These findings indicate the very efficient presentation of mIL-15 by membrane-bound mIL-15Rα to mRβ/γ on the same cell surface (cis presentation). Similarly, Dubois et al. (14) previously suggested that cells expressing Rβ but not IL-15Rα react to IL-15 by trans presentation with cellular IL-15Rα, whereas cells expressing both Rβ and IL-15Rα react to IL-15 by cis presentation. However, normal CD8 T cells and some T cell lines show no evidence of the cis presentation of mIL-15, even though they express all the α/β/γ receptor components. The analysis of the surface expression levels of mIL-15Rα and mRβ on these cell lines suggests that the expression level of mIL-15Rα plays a critical role in cis presentation. Increased expression of mIL-15Rα allows these cells to use cis presentation of mIL-15 to proliferate in vitro. Therefore, it is clear that the amount of mIL-15Rα expressed determines whether the cells require the trans presentation of mIL-15 or can use surface mIL-15Rα for the cis presentation of mIL-15. A similar receptor–ligand system, in which a specific receptor captures the ligand to present it to the signaling receptor, has been reported for IL-6 (25), in which signaling is mediated by a soluble capture receptor (IL-6R) and a membrane-bound signaling receptor (gp130), as well as two membrane-bound receptors. It is unique to the IL-15 system that the three kinds of interaction show different sensitivities: the cells expressing Rβ/γ and no or low levels of IL-15Rα respond to IL-15 with low sensitivity; these cells react to IL-15–IL-15Rα complex (trans presentation) with medium sensitivity; and the cells expressing Rβ/γ and high levels of IL-15Rα respond to IL-15 (cis presentation) with high sensitivity. The importance of cis presentation in the mouse IL-15 system remains unclear and requires further study.

Crystallographic analysis of the IL-15–IL-15Rα complex has provided no structural basis for the cis and trans presentation of IL-15 (17, 24). In both the human and mouse structures, the importance of the Sushi domain of IL-15Rα in binding to IL-15 has been clearly shown, but there is no information concerning the stalk portion of the receptor. However, the C terminus of soluble IL-15Rα is oriented in such a way that both cis and trans presentation could occur with a flexible stalk region (17). A recent study has shown that the 13-amino-acid hinge region adjacent to the Sushi domain contributes to the high affinity of IL-15Rα for IL-15 and that this region is important for the agonistic or antagonistic action of soluble IL-15Rα on Rβ/γ- or Rα/β/γ-expressing cells, respectively (26). It should be noted that the soluble IL-15Rα used in our study contained the full-length ectodomain, so that the affinity of our soluble IL-15Rα for IL-15 was high enough to show its biological activity. We are currently analyzing the relationship between the flexibility of the stalk region of IL-15Rα and its capacity to present IL-15 in cis and trans. This analysis, together with a comparison of the soluble and receptor-bound forms of IL-15, may clarify the biological difference between the cis and trans presentation of IL-15 and the dosage requirement of IL-15Rα for cis presentation.

A study using FDC3 cell lines expressing human R (hRβ alone, or hRβ and hIL-15Rα) has demonstrated the cis and trans presentation of hIL-15, as in the murine system. In this study, we used the same cell line to monitor, although indirectly, the expression levels of receptors introduced by retrovirus-mediated gene transfer. Our results suggest that the cis presentation of hIL-15 by hIL-15Rα does not require high-level expression of this receptor on the cell surface. Furthermore, the comparison of the FDC3 cell lines expressing human and murine receptors revealed that the cells expressing hRβ or hRα/hRβ proliferate at much lower concentration of hIL-15 than that of the same cell line expressing mRβ or mRα/mRβ in response to mIL-15, respectively.

In contrast with FDC3 cell line expressing human IL-15 receptors, proliferative response of primary human CD8 T cell to IL-15 was not affected by an addition of soluble hIL-15Rα in vitro. It is unclear why soluble hIL-15Rα has neither positive nor negative effect on human CD8 T cells. However, several possibilities may explain this phenomenon. Due to the high-affinity interactions in the human system, in vitro-cultured human CD8 T cells may be using cis presentation to respond to hIL-15, and addition of IL-15Rα does not show any enhancing effect. Also, the overall response of primary cultured T cells to IL-15 is not as vigorous as that of FDC3 cells expressing high level of IL-15Rα-chain. In such a condition, cis to trans presentation occurs in the narrow IL-15 dose range making it difficult to see the inhibitory effect of soluble IL-15Rα. Alternatively, it is possible that normal human CD8 T cells, but not established in vitro-cultured mouse cell lines expressing human receptors, may require neither cis nor trans presentation, and addition of soluble IL-15Rα has no effect. Human primary CD8 T cells with different level of expression of the receptors are necessary to test these possibilities but are not possible to establish with current technology. It is also possible that primary human CD8 T cells exhibit strong cross-cellular trans presentation, which is dependent on cell density. We used same cell density in human and mouse T cell experiments and found that addition of soluble mIL-15Rα enhanced the proliferative response of mouse CD8 T cell, suggesting that the trans presentation by soluble IL-15Rα is more efficient than cross-cellular trans presentation at this cell density. Thus, we think cross-cellular trans presentation was not dominant in our experimental condition, even though it might occur at some level. To test the efficiency of cross-cellular trans presentation, human CD8 T cells lacking either the expression of hIL-15Rα or the expression of hRβ are essential, but establishing such cells is technically challenging.

At present, the roles of cis and trans presentation in the generation and maintenance of human CD8 T cells in vivo is not understood. Cell types, such as NK and NKT cells, that do not express IL-15Rα definitely require trans presentation in the human system. However, the results presented in this study raise the possibility that the biological role of the IL-15–IL-15R interaction...
revealed by studies of the murine system may not be applicable to the biology of human CD8 T cells in vivo. Therefore, a new approach is required to understand how IL-15 mediates its function in the generation and maintenance of human memory CD8 T cells in vivo. Understanding the mechanisms underlying the generation and maintenance of human CD8 T memory cells should provide new insights and approaches to the production of effective vaccines for infectious diseases in humans.

Disclosures

The authors have no financial conflicts of interest.

References


Supplementary Figure Legends

**Supplementary Figure 1.** *Trans* presentation of IL15 by cellular mIL15Rα enhanced the proliferation of FDC3 cells expressing mRβ. Thymidine uptake was measured in FDC3 cells expressing mRβ (open circles), FDC3 cells expressing mIL15Rα (open triangles), a mixed culture of FDC3 cells expressing mRβ and FDC3 cells expressing mIL15Rα (closed circles), and a mixed culture of FDC3 cells expressing mRβ and untransfected FDC3 cells (closed triangles). The data shown are representative results of more than three experiments.

**Supplementary Figure 2.** The complex of soluble mIL15Rα–mIL15 enhanced the proliferation of FDC3 cells expressing mRβ, similar to their response to mIL15 + soluble mIL15Rα. Thymidine uptake was measured in FDC3 cells expressing mRβ stimulated with either mIL15 alone (open circles), mIL15 + soluble mIL15Rα (closed circles), or with a purified preformed complex of soluble mIL15Rα and mIL15 (closed triangles). When the cells were stimulated with the complex, the amount of protein was adjusted based on the amount of mIL15. The data shown are representative results of
more than two experiments.

**Supplementary Figure 3.** The enhanced mIL15 response induced by soluble mIL15Rα was observed in both activated and naive CD8 T cells. Thymidine uptake was measured in ConA-stimulated or naïve CD8 T cells cultured with either mIL15 alone (open circles) or mIL15 + soluble mIL15Rα (closed circles). The data shown are representative results of more than two experiments.
Supplementary Figure S1 Kanagawa et al.
Supplementary Figure S2 Kanagawa et al.

Thymidine uptake (cpm/well) vs. mouse IL-15 (ng/ml)

- mIL-15
- mIL-15 + smIL15Rα complex
- mIL-15 + smIL15Rα
- mIL-15 + mIL-15Rα

y-axis: Thymidine uptake (cpm/well)

x-axis: mouse IL-15 (ng/ml)
Supplementary Figure S3 Kanagawa et al.
Supplementary Table I. Summary of the Responses of FDC3 Cells to Mouse and Human IL15

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<th>Receptor expressed by FDC3</th>
<th>IL15</th>
<th>Soluble IL15Rα</th>
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<tr>
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Mouse IL15 did not stimulate FDC3 cells expressing human receptors.