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MHC Class I and TCR Avidity Control the CD8 T Cell Response to IL-15/IL-15Rα Complex

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Abstract

IL-15 operates via a unique mechanism termed transpresentation. In this system, IL-15 produced by one cell type is bound to IL-15Rα expressed by the same cell and is presented to apposing cells expressing the IL-15Rβ/γC complex. We have shown that administering soluble IL-15Rα complexed with IL-15 can greatly enhance IL-15 activity. We now show that the naive CD8 T cell response to exogenous IL-15/IL-15Rα complex is MHC class I dependent. In the absence of β2 microglobulin, naive CD8 T cells scarcely proliferated in response to IL-15/IL-15Rα complex, whereas memory cells proliferated, although to a lesser extent, compared with levels in control mice. The loss of β2m or FcRn slightly reduced the extended half-life of IL-15/IL-15Rα complex, whereas FcRn deficiency only partially reduced the naive CD8 T cell proliferative response to IL-15/IL-15Rα complex. In addition, we demonstrated a link between TCR avidity and the ability of a T cell to respond to IL-15/IL-15Rα complex. Thus, T cells expressing low-avidity TCR responded poorly to IL-15/IL-15Rα complex, which correlated with a poor homeostatic proliferative response to lymphopenia. The inclusion of cognate peptide along with complex resulted in enhanced proliferation, even when TCR avidity was low. IL-15/IL-15Rα complex treatment, along with peptide immunization, also enhanced activation and the migratory ability of responding T cells. These data suggest that IL-15/IL-15Rα complex has selective effects on Ag-activated CD8 T cells. Our findings have important implications for directing IL-15/IL-15Rα complex-based therapy to specific Ag targets and illustrate the possible adjuvant uses of IL-15/IL-15Rα complex.

Overlapping factors regulate the naive and memory CD8 T cell pools in the periphery. Naive CD8 T cells, but not memory CD8 T cells, require contact with self-peptide presented by MHC class I molecules, whereas both populations require IL-7 for their survival (1–5). Furthermore, memory CD8 T cells require IL-15 for their continued homeostatic proliferation in an immunosufficient background (6). IL15$^{-/-}$ and IL-15Rα$^{-/-}$ mice have a significantly smaller naive CD8 T cell compartment, possibly due to reduced CD8 T cell thymic development and survival in the periphery (7). These requirements for maintenance

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and survival are paralleled when T cells are placed in an immunodeficient environment. Specifically, naive CD8 T cells require host MHC class I and IL-7 expression to undergo acute homeostatic proliferation in irradiated or RAG-deficient hosts (5, 8–11). IL-15 is not required for initiating naive CD8 T cell acute homeostatic proliferation, but rather it helps to sustain the process at later time points (12). In contrast, memory CD8 T cells proliferate in the absence of MHC class I, but they require IL-7 and IL-15 for their maximum proliferation in a lymphopenic setting (3, 5).

The identification of the mechanism of IL-15–mediated activity, namely transpresentation (13, 14), led to the use of precomplexed IL-15 and soluble IL-15Rα as immunostimulants (15–17). Thus, presentation of rIL-15 bound with high affinity to soluble rIL-15Rα drives robust activation of cells expressing IL-2/15Rβ and the γC in vitro and in vivo. Memory CD8 T cells and NK cells respond most vigorously, and IL-15/IL-15Rα (IL-15 complex) treatment augments tumor clearance (16, 18, 19).

Surprisingly, naive CD8 T cells also respond to IL-15 complex, despite their low expression level of IL-15Rβ (11, 16). Of note, the naive CD8 T cell response to IL-15 complex mimics the response to cognate Ag in that the naive CD8 T cell population undergoes an expansion phase and acquires an activated phenotype and effector functions, including the ability to secrete IFN-γ and mediate Ag-specific cytolytic function. Importantly, no exogenous Ag was required to initiate this response. Similarly, naive CD8 T cells undergoing acute homeostatic proliferation in immunodeficient hosts acquire a memory phenotype with increased expression of CD44, IL-2/IL-15Rβ and γC, Ly6C, and CD69 (10, 20, 21). Some initial studies stated that naive CD8 T cells proliferating in a lymphopenic environment do not acquire effector function after transfer (22), whereas others showed the acquisition of potent effector responses (20, 21). However, this phenomenon requires multiple rounds of division and develops relatively late posttransfer, which may explain the differences between these studies. In addition, acute homeostatic proliferation results in the appearance of long-term stable memory-phenotype CD8 T cells (23, 24). Thus, acute lymphopenic proliferation and IL-15/IL-15Rα–induced proliferation of naive CD8 T cells result in the acquisition of memory phenotypes, although IL-15 complex stimulation operates with more rapid induction of proliferation (16).

Although acute slow homeostatic proliferation occurs without the addition of cognate Ag, the process is dependent on the presence of MHC class I presenting self-peptide (8, 9, 25). However, exogenous peptides derived from normal flora drive a subset of naive polyclonal T cells to undergo rapid homeostatic proliferation (26). Furthermore, although some naive TCR transgenic T cells, such as SIINFEKL-specific OT-I TCR transgenic CD8 T cells, undergo massive proliferation when placed in a lymphopenic environment, this is not the case with all naive CD8 T cells (9–11). This effect seems to be due, in part, to differences in TCR triggering, whether due to TCR density, negative-regulator CD5 levels, TCR avidity, or TCR promiscuity (8, 9, 27–29). These findings indicate that the strength of the TCR stimulus is directly related to the extent of proliferation in response to lymphopenia (10).

Because naive CD8 T cells responding to IL-15 complex seem to undergo a similar activation process as cells responding to lymphopenia (16, 21), we set out to compare the requirements for induction of T cell activation by these conditions. The results indicated that IL-15 complex-driven CD8 T cell activation operates through TCR signaling and not solely through cytokine receptors. Understanding the mechanism through which IL-15 complex modulates the immune system holds implications for the proper application of IL-15 immunotherapy.

Materials and Methods

Mice

C57BL/6-CD45.2 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and the Charles River-National Cancer Institute. C57BL/6-CD45.1 mice were purchased from Charles River-National Cancer Institute (Frederick, MD). B10.D2 mice (recipients of C4 transfers) were purchased from The Jackson Laboratory. The OT-I mouse line was maintained as a C57BL/6-CD45.1 line on a *Rag*$^{-/-}$ background. FcRn$^{-/-}$ mice were a generous gift from Dr. Lynn Puddington (University of Connecticut Health Center) (30). β2m$^{-/-}$ mice and their respective C57BL/6-CD45.2 controls were purchased from Taconic Farms (Germantown, NY). The F5 (31) and C4 (32) mice were generously provided by Dr. Linda Cauley and Dr. Adam Adler, respectively (University of Connecticut Health Center). All procedures were carried out under National Institutes of Health guidelines and were approved by the University of Connecticut Health Center Animal Care Committee.

IL-15 treatment

Human IL-15 (hIL-15) was generously provided by Amgen (Thousand Oaks, CA). Mouse rIL-15Rα–Fc chimeric molecule was purchased from R&D Systems (Minneapolis, MN). hIL-15 and mouse rIL-15Rα–Fc, both suspended in PBS, were mixed and incubated for 30 min at 37°C. Each mouse, unless specifically noted, received 2.5 μg IL-15 alone or pre-complexed with 15 μg mouse rIL-15Rα–Fc in 200 μl PBS i.p.

Purification and adoptive transfer of cells

Single-cell suspensions were created in HBSS by homogenizing spleens and lymph nodes using frosted glass slides. RBCs were lysed using Tris-ammonium chloride buffer, and cells were filtered through Nitex. CD44^{lo} CD8 T cells (includes TCR transgenic T cells) were enriched using a combination of anti–CD44-biotin and antibiotin microbeads (Miltenyi Biotec, Auburn, CA), as previously described (33). In experiments comparing the proliferation of CD44^{lo} and CD44^{hi} polyclonal CD8 T cells, both populations were purified by flow cytometry using a FACSVantage SE (BD Biosciences, San Jose, CA). For CFSE labeling, cells were incubated for 10 min at 37°C with CFSE (0.01 mM; Invitrogen, Carlsbad, CA), the reaction was quenched with HBSS with 5% FCS (34), and the cells were washed twice. CFSE-labeled cells were resuspended in PBS and injected i.v. into congenic mice. Unless otherwise specified in the figure legends, the number of cells transferred was between 1×10^5 and 3×10^5. For infections, 24 h post–OT-I transfer mice were infected i.v. with 1×10^5 vesicular stomatitis virus expressing OVA (VSV-OVA).

Isolation of cells from tissues

Using frosted glass slides, spleen and lymph nodes were homogenized into single-cell suspensions in HBSS/5% bovine serum. Lungs, liver, and brains were minced into small pieces and incubated with collagenase buffer (RPMI 1640, 2% FCS, containing HEPES, pen-strep, glutamine, gentamicin, $CaCl_2$, $MgCl_2$, and collagenase [100 U/ml; Invitrogen, Carlsbad, CA]) for 1 h (lung and liver) or 30 min (brain) at 37°C under agitation. At the end of incubation, remaining tissue pieces were crushed through 70-μm nylon mesh filter (BD Biosciences). The resulting cells were pelleted and re-suspended in 44% Percoll buffer (GE Life Sciences, Piscataway, NJ) and underlain with 67% Percoll buffer. Percoll gradients were centrifuged at 1700 × *g*, and cells at the Percoll gradient interface were extracted, washed, and resuspended in HBSS/5% bovine serum/HEPES, pen-strep, glutamine, gentamicin.

Flow-cytometric analysis

Cells were isolated at the indicated times and analyzed for the presence of donor cells using CD45 or Thy1 allele status and their expression of surface markers and CFSE intensity. For staining, lymphocytes were suspended in PBS/0.2% BSA/0.1% NaN_3 (FACS buffer) at a concentration of 5×10^6/100 μl and stained with indicated Abs at 4°C for 20 min, washed, and fixed in PBS with 2% paraformaldehyde. Samples were acquired on a FACS-Calibur or LSR II (BD Biosciences). Data were analyzed using FlowJo Software (Tree Star, Ashland, OR).

Peptide activation and treatments

Congenic naive T cells were transferred into appropriate hosts (OT-I and F5 into B6 hosts) that were subsequently treated as described in the figure legends. For peptide treatments, mice received 100 μg of the respective peptide, unless stated otherwise. The peptide sequences were as follows from N terminus to C terminus: SIINFEKL (OT-I–specific epitope presented by H-$2K^b$) and ASNENMETM (F5-specific epitope from influenza virus NP presented by H-$2D^b$). For combination peptide and IL-15 complex treatment, peptides were added to the mixture of IL-15 and IL-15Rα–Fc (as described above) prior to injection. All treatments were given i.p.

Serial adoptive transfers and T cell-migration assay

We performed serial adoptive transfers to isolate OT-I T cells subjected to different activating conditions and to test their migratory potential. Naive $CD44^{lo}$ CD45.1 OT-I cells were transferred into naive CD45.2 B6 hosts; 1 d later, hosts were injected with IL-15 complex, a mixture of 100 μg SIINFEKL and IL-15 complex, or VSV-OVA. Four days posttreatment, spleens were harvested and processed into single-cell suspensions. Splenocytes were enriched for activated CD45.1 donor OT-I cells using MACS technology. In short, single-cell suspensions were stained with CD45.1 biotin and enriched for CD45.1 OT-I using antibiotin microbeads and MACS LS separation columns, according to Miltenyi Biotec protocols. Approximately 1×10^6 enriched activated CD45.1 OT-I cells, mixed with CD45.2 naive B6 splenocytes to equalize the total number of cells transferred into each host, were transferred into secondary CD45.2 B6 hosts. For the naive OT-I control, naive $CD44^{lo}$ OT-I cells were isolated as described above, but hosts were left untreated. Two days following secondary OT-I transfer, cells from spleen, peripheral lymph nodes (pLNs) (inguinal, brachial, axillary, and cervical lymph nodes), lung, and liver were isolated and processed as described above. Presence of CD45.1 donor OT-I cells was analyzed by flow cytometry. The percentage of donor cells in each tissue was formulated as follows: the number of CD45.1 donor OT-I cells in a particular tissue was divided by the total number of CD45.1 donor OT-I cells in the spleen, pLNs, liver, and lung and then multiplied by 100.

Results

Naive CD8 T cell response to IL-15 complex is MHC class I dependent

As we previously illustrated, naive CD8 T cells acquire an activated phenotype and effector function in response to IL-15 complex (16). Although cognate Ag is not required for these effects, it is not known whether the response is MHC class I dependent. To test this possibility, we transferred CFSE-labeled $CD44^{lo}$ CD45.1 naive OT-I TCR transgenic CD8 T cells (Fig. 1*A*) into sublethally irradiated (500 rad) CD45.2 B6 and MHC class I-deficient ($\beta2m^{-/-}$) mice on day −1. Hosts were irradiated to prevent rejection of donor cells in $\beta2m^{-/-}$ mice. On day 0, B6 and $\beta2m^{-/-}$ mice were treated with PBS or IL-15 complex (2.5 μg IL-15 and 15 μg sIL-15Rα–Fc; doses used for all experiments), and splenocytes were analyzed on day 4 posttreatment for OT-I proliferation. As expected, the naive $CD44^{lo}$ OT-I

cells underwent homeostatic proliferation in irradiated B6 hosts, and this proliferation was substantially augmented with IL-15 complex treatment (Fig. 1*A*). In contrast, in untreated irradiated β2m$^{-/-}$ hosts, naive OT-I CD8 T cells did not proliferate. With IL-15 complex treatment, only a low level of proliferation was induced in β2m$^{-/-}$ mice, as evidenced by blunted CFSE dilution and reduced cell numbers (Fig. 1*A*). The minor response detected in β2m$^{-/-}$ hosts may be due to the interaction of OT-I cells with other MHC class I^{+} donor cells or with MHC class II present in the host (29, 35). Thus, the naive CD8 T cell response to IL-15 complex was largely dependent on the presence of MHC I.

Contrary to naive CD8 T cells, the proliferation of CD44^{hi} CD8 T cells in a lymphopenic environment is not MHC class I dependent (2, 3). To test whether this was also the case with IL-15 complex, sorted CFSE-labeled CD44^{hi} Thy1.1 and CD44^{lo} CD45.1 polyclonal CD8 T cells were transferred together into sublethally irradiated (550 rad) CD45.2/Thy1.2 B6 and β2m$^{-/-}$ hosts on day −1. On day 0, B6 and β2m$^{-/-}$ mice were treated with PBS or IL-15 complex, and splenocytes were analyzed for the proliferation of donor cells on day 4. Consistent with the OT-I data, the proliferation of CD44^{lo} polyclonal CD8 T cells was greatly reduced in β2m$^{-/-}$ hosts compared with B6 hosts (Fig. 1*B*). In contrast, treatment with IL-15 complex substantially enhanced proliferation of memory-phenotype cells in B6 hosts and, to a lesser extent, in β2m$^{-/-}$ hosts (Fig. 1*C*). The results indicated that the response of naive CD8 T cells to IL-15 complex had a greater dependence on MHC class I than did memory-phenotype cells, suggesting that complex treatment mimicked events seen in a lymphopenic situation (3, 8, 10).

FcRn partially regulates IL-15/IL-15Rα complex pharmacology

Although β2m$^{-/-}$ mice lack MHC class I expression, β2m is also required for expression of the neonatal Fc receptor FcRn (36). FcRn plays an important role in IgG catabolism through IgG uptake and recycling, such that FcRn deficiency results in reduced IgG half-life. Because the IL-15Rα component of IL-15 complex contains an IgG Fc region, we investigated whether β2m expression affected complex half-life. To this end, B6 and β2m$^{-/-}$ hosts were administered equal amounts of IL-15 complex, and serum IL-15 levels were measured at various times after treatment by ELISA (16). At early time points, more IL-15 was detected in the serum of β2m$^{-/-}$ mice compared with control mice (Fig. 2*A*). However, IL-15 decayed more rapidly in β2m$^{-/-}$ mice, and the total amount of available IL-15 was ~60% of that in B6 mice, as measured by calculating the area under each curve. The role of FcRn in this phenomenon was directly assessed using FcRn$^{-/-}$ hosts. IL-15 serum half-life exhibited similar kinetics in FcRn$^{-/-}$ hosts as in β2m$^{-/-}$ hosts, although no early spike in IL-15 levels was noted as in β2m$^{-/-}$ mice (Fig. 2*B*). As seen in β2m$^{-/-}$ mice, we noted that the total amount of available IL-15 in FcRn$^{-/-}$ mice was ~60% of that in B6 mice. We also measured the proliferation of naive OT-I cells in sublethally irradiated B6 and FcRn$^{-/-}$ hosts. Without complex treatment, OT-I cells divided equally well in sublethally irradiated FcRn$^{-/-}$ and B6 hosts (Fig. 2*C*). However, OT-I cell proliferation was partially reduced in IL-15 complex-treated FcRn$^{-/-}$ mice compared with B6 hosts, which may be explained by the reduction in IL-15 half-life and total availability (Fig. 2*B*). Nonetheless, proliferation of naive OT-I cells in FcRn$^{-/-}$ mice was substantially greater than that obtained in β2m$^{-/-}$ mice (Fig. 1*A*). Thus, although the lack of FcRn influenced the naive CD8 T cell response in β2m$^{-/-}$ mice, the majority of IL-15 complex-driven proliferation was mediated via MHC class I recognition. Furthermore, the reduced in vivo half-life of IL-15 complex in β2m$^{-/-}$ mice may explain the decreased response of CD44^{hi} polyclonal CD8 T cells to IL-15 complex in these mice.

TCR avidity for self-MHC controls responsiveness to IL-15 complex

Not all naive CD8 T cells respond equally well to a lymphopenic environment (4), and this finding has been linked to TCR avidity (28). Because the CD8 T cell response to IL-15 complex was also MHC class I dependent, it was of interest to test whether relative TCR avidities affected the response. We first examined whether the response of naive CD8 T cells to IL-15/IL-15Rα complex treatment exhibited heterogeneity. CFSE-labeled CD45.1 $CD44^{lo}$ naive polyclonal B6 CD8 T cells were transferred into normal CD45.2 B6 hosts on day −1 that were then treated with PBS or IL-15 complex on day 0. Concurrently, CFSE-labeled CD45.1 $CD44^{lo}$ naive polyclonal CD8 T cells were also transferred into CD45.2 B6 hosts sublethally irradiated (550 rad) 1 d earlier. At 4–5 d posttreatment, splenocytes were examined for CFSE dilution. A robust proliferative response was seen only when the mice were exposed to IL-15 complex (Fig. 3*A*). Interestingly, the polyclonal CD8 T cell population divided in an asynchronous fashion, in contrast to OT-I cells (Fig. 1), with some cells undergoing extensive division and others barely dividing. Thus, not all naive endogenous CD8 T cells responded to IL-15 complex to the same extent. Similarly, although the proliferative response was not as robust as in IL-15 complex-treated hosts, polyclonal CD8 T cells also exhibited an asynchronous division in sublethally irradiated hosts (Fig. 3*A*).

To more closely examine how TCR avidity may influence the response to IL-15 complex, we compared the response of naive TCR transgenic CD8 T cells of different specificities and distinct TCR avidities (28). To this end, naive CFSE-labeled $CD44^{lo}$ CD45.1 OT-I (specific for SIINFEKL peptide presented by $H\text{-}2K^b$), F5 (specific for influenza virus NP peptide presented by $H\text{-}2D^b$), and C4 (specific for influenza virus HA peptide presented by $H\text{-}2K^b$) TCR transgenic CD8 T cells were transferred into CD45.2 B6 or B10.D2 (for C4) mice on day −1. On day 0, B6 hosts were treated with PBS or IL-15 complex. TCR transgenic T cells were also transferred into CD45.2 B6 mice sublethally irradiated (550 rad) 1 d in advance. On day 5 posttreatment, donor cells were analyzed for CFSE dilution. In an immunodeficient environment (sublethally irradiated hosts), OT-I and C4 cells divided extensively, whereas F5 cells did not divide (Fig. 3*B*). Thus, the TCR avidity of F5 CD8 T cells is insufficient to drive homeostatic proliferation. Interestingly, the response of OT-I, F5, and C4 TCR transgenic CD8 T cells to IL-15 complex paralleled the response to lymphopenia. This was not due to differences in CD122 or CD132 expression levels between the TCR transgenic T cells (data not shown). Compared with lymphopenia-driven expansion, proliferation was better with IL-15 complex treatment in all cases, with some limited proliferation observed with F5 T cells (Fig. 3*B*).

IL-15 complex enhances the naive CD8 T cell response to cognate peptide

Providing naive CD8 T cells their cognate peptide during acute homeostatic proliferation enhances their proliferative response (8, 10). Therefore, we examined whether IL-15 complex treatment, along with cognate peptide, would further enhance the naive CD8 T cell response to IL-15 complex. CFSE-labeled CD45.1 $CD44^{lo}$ OT-I and F5 cells were transferred into separate CD45.2 B6 hosts on day −1. On day 0, hosts were treated with PBS, cognate peptide, IL-15/IL-15Rα, or cognate peptide and IL-15/IL-15Rα. On day 5, donor cell division was measured by flow cytometry. Peptide alone induced significant proliferation in F5 cells, whereas IL-15 complex was a poor inducer of cell division. However, the F5 cell response was greatly enhanced when IL-15 complex and cognate peptide were administered (Fig. 4*A*). Similar results were obtained with OT-I cells, although their response to IL-15 complex alone was much greater than that of F5 cells (Fig. 4*B*). We also examined the accumulation of the different cell populations in each case. Peptide treatment alone, although inducing cell division, did not result in significant accumulation of responding cells, likely due to deletion (Fig. 4). Treatment with the combination of cognate

peptide and IL-15 complex seemed to be synergistic, resulting in substantial accumulation of cells. In additional studies in which OT-I cells and F5 cells were cotransferred, OT-I cells outcompeted F5 in each of the responses (Supplemental Fig. 1). This finding further supported the concept that TCR avidity was controlling the response to IL-15/IL-15Rα complex. Nevertheless, these results suggested that IL-15 complex acts selectively on Ag-activated CD8 T cells and could serve as an adjuvant for Ag-specific responses. Furthermore, the cell-accumulation data illustrated that naive CD8 T cells normally unresponsive to IL-15 complex were more responsive when TCR was engaged.

We also examined the effect of peptide and IL-15 complex treatment on T cell migration to nonlymphoid tissues. CD45.1 $CD44^{lo}$ naive OT-I cells were transferred into CD45.2 B6 hosts on day −1, and on day 0 these mice were treated with PBS, SIIN-FEKL peptide, IL-15 complex, or SIINFEKL peptide and IL-15 complex (peptide/IL-15 complex). Five days posttreatment, mice were sacrificed, and various tissues were examined for the presence of the OT-I donor population. IL-15 complex treatment increased OT-I cell numbers in the lung, as well as in the spleen. An even larger OT-I donor population was present in both tissues of mice treated with peptide/IL-15 complex compared with IL-15 complex treatment alone (Fig. 5). A similar pattern was seen in the liver (data not shown). Comparable results were obtained when F5 cells were transferred and the treatments administered, although, as expected, fewer total F5 cells compared with OT-I cells, were recovered from the organs (Supplemental Fig. 2). When the brain, a more immunoprivileged tissue (37, 38), was examined, very few OT-I CD8 T cells were present in control, peptide- or IL-15 complex-treated mice. In contrast, a sizable population of CD8 T cells was present in the brains of peptide/IL-15 complex-treated mice (Fig. 5).

The previous data suggested that IL-15 complex treatment, along with peptide immunization, enhanced lymphocyte migration. Therefore, we examined the expression of various adhesion molecules after treatment. All treatments uniformly upregulated CD11a (Fig. 5*B*). Treatment with IL-15 complex alone weakly upregulated CD43 and did not induce CD62L downregulation, but it modestly enhanced CD44 expression. Peptide treatment alone strongly induced CD43 expression, modestly downregulated CD62L expression, and greatly upregulated CD44 levels. Peptide/IL-15 complex was the most effective at CD43 induction and CD62L downregulation, and it strongly induced CD44 expression. Thus, IL-15 complex enhanced the proliferative response to cognate Ag, and the combination of IL-15 complex and Ag also resulted in the modulation of adhesion receptors involved in migration and enhanced function.

We performed serial adoptive transfers to formally test whether IL-15 complex altered the migration potential of the responding CD8 T cells. CD45.1-naive $CD44^{lo}$ OT-I cells were transferred into CD45.2 B6 hosts on day −1 and then treated with IL-15 complex, peptide/IL-15 complex, or VSV-OVA on day 0. VSV-OVA infection causes similar changes in cell-surface phenotype as does peptide/IL-15 complex (data not shown) and was used as a positive control for migration potential to tertiary sites. On day 4 posttreatment, splenocytes were harvested and enriched for donor OT-I cells. A total of 1×10^6 CD45.1 OT-I cells from each group, including naive $CD44^{lo}$ OT-I cells as a control, were transferred into secondary CD45.2 B6 hosts. Two days after secondary transfer, hosts were sacrificed, and the presence of donor OT-I cells was determined in several tissues (Fig. 6). Naive OT-I cells were mainly found in the spleen and pLNs. In contrast, VSV-OVA–experienced OT-I cells were found in the spleen, as well as in the lungs and liver, with few present in the pLNs. Cells from IL-15 complex-treated mice behaved similarly to naive CD8 T cells, whereas the migration pattern of peptide/IL-15 complex-treated cells resembled that of virus-activated OT-I cells. Overall, the data support the notion that IL-15 complex treatment can augment

CD8 T cell activation and enhance migratory capabilities, both of which are necessary outcomes of effective immunotherapy.

Discussion

Our previous work highlighted the intriguing ability of IL-15Rα-complexed IL-15, but not IL-15 alone, to activate naive CD8 T cells (16). This response was Ag independent, but we now show that the process required expression of MHC class I. Furthermore, the ability of a naive CD8 T cell to respond to IL-15 complex correlated with TCR avidity for self-peptide:MHC class I. Intriguingly, the response was augmented, as well as altered, by providing a stronger TCR stimulus. In particular, IL-15 complex induced a lymphoid-migration profile, whereas agonist peptide/IL-15 complex induced a nonlymphoid-migration profile. These latter results point out some important considerations for the use of IL-15 complex as a therapeutic and may help to explain some recent findings.

Recent work revealed similarities in the naive CD8 T cell response to IL-15 complex and IL-2α/IL-2 complex (IL-2 complex). Similar to the effects of IL-15 complex treatment (16), naive CD8 T cells treated with IL-2 complex in vivo proliferate, acquire an activated/memory phenotype, produce effector cytokines, and kill in an Ag-specific manner (39). In apparent contrast to our findings, IL-2 complex-driven naive CD8 T cell activation is classical MHC class I independent (39). However, this study used H-2K^{b} and H-2D^{b} double-deficient mice, which still express other MHC class I molecules. Indeed, another report found that the CD8 T cell response to IL-2 complex is MHC class I dependent when using K$^{b-/-}$ D$^{b-/-}$ β2m$^{-/-}$ triple-knockout mice (35), ensuring that classical and nonclassical MHC class I molecules are absent. An additional study found that in CD132$^{-/-}$ (γC$^{-/-}$) mice, in which IL-2 and IL-15 levels are abnormally high, naive CD8 T cell proliferation is only modestly affected by the absence of MHC class I (40), perhaps as a result of the combined effects of both cytokines. Overall, these findings further illustrate the close relationship between factors controlling lymphopenia-driven and cytokine complex-driven naive CD8 T cell activation.

One caveat with the use of β2m$^{-/-}$ mice to study the response to an IgG-Fc region-containing molecule is the potential role of FcRn in IgG catabolism (36). This issue could be a confounding factor in our studies and that of Cho et al. (35). In fact, we found that the in vivo half-life of IL-15 was modestly and similarly reduced in β2m$^{-/-}$ and FcRn$^{-/-}$ mice. However, IL-15 complex-driven naive CD8 T cell proliferation remained robust in FcRn$^{-/-}$ mice, whereas it was substantially diminished in β2m$^{-/-}$ mice. Thus, the absence of FcRn cannot account for the lack of proliferation seen in β2m$^{-/-}$ mice. Not surprisingly, the activity of IL-2/α–IL-2 was also recently shown to be dependent, in part, on FcRn (41).

Our data indicated that not all naive CD8 T cells responded with the same intensity to IL-15 complex. A similar spectrum of proliferation is seen when naive CD8 T cells are placed in a lymphopenic environment (9). Recent work noted that TCR avidity for self-peptide:MHC class I regulates the CD8 T cell response to lymphopenia. Thus, in a lymphopenic environment, T cells with high-avidity TCRs proliferate more vigorously than do T cells with lower-affinity TCRs (28). Our results demonstrated a direct correlation between the relative ability of a naive CD8 T cell to respond to IL-15 complex or to lymphopenia. Furthermore, the response to IL-15 complex positively correlated with TCR avidity, as measured by acute homeostatic proliferation. Greater TCR signaling may confer heightened responsiveness to homeostatic cytokines, such as IL-7, especially in more competitive situations (28, 42). Thus, the cytokine-driven response to lymphopenia also requires a level of tonic TCR signaling, although there is some evidence of TCR-independent cytokine-mediated proliferation in lymphopenic settings (29). There is also a link between IL-15

adjuvanticity and TCR avidity, because IL-15 administration causes the outgrowth of higher-avidity CTLs in response to infection (43). Our cotransfer studies reiterated the concept that high-avidity clones may outpace low-avidity clones in response to IL-15 complex in the presence or absence of Ag. Given these findings, IL-15 complex may operate as an effective immunotherapy by preferentially stimulating the expansion of high-avidity naive or effector CD8 T cells, in part through TCR- and MHC-mediated signals.

Of particular interest was our finding that IL-15 complex and peptide/IL-15 complex treatments caused distinct changes in the expression of surface markers associated with migration. Although complex and peptide/IL-15 complex caused upregulation of CD11a and CD44, only peptide/IL-15 complex caused significant down-regulation of CD62L and upregulation of CD43. CD43 plays a role in the migration of effector cells from secondary lymphoid tissues to tertiary sites of inflammation, in particular the brain (44, 45). Furthermore, the concomitant downregulation of CD62L and up-regulation of CD44 also promote migration to tertiary sites (46–48). Overall, the changes in surface expression of the receptors correlated with the migratory behavior of the cells. CD8 T cells activated by peptide/IL-15 complex treatment migrated more readily to nonlymphoid tissues compared with cells activated with IL-15 complex alone. The efficacy of IL-15 complex in tumor immunotherapy may hinge, in some cases, on the ability of IL-15 complex to alter effector cell migration. For example, partial regression of solid tumors is induced by IL-15 complex by activation of intratumor tumor-specific CD8 T cells (19). The investigators noted that IL-15 complex treatment more effectively stimulated intratumor CD8 T cells than peripheral CD8 T cells, and IL-15 complex treatment did not enhance CD8 T cell migration into tumors. Given our current results, the robust response of intratumor CD8 T cells may be explained by access and interaction of intratumor, but not peripheral, CD8 T cells with cognate Ag. Without access to tumor Ag, IL-15 complex-activated tumor-specific CD8 T cells may not efficiently enter pre-existing tumors because of the lack of effective modulation of homing receptors that promote extralymphoid migration. Thus, stronger TCR stimulation, in conjunction with IL-15R signaling, may help to spur migration of CTLs into tertiary tissues and, possibly, tumors.

Overall, our findings highlight the ability of IL-15 complex to specifically induce activation and proliferation of naive CD8 T cells expressing high-avidity TCRs. This process is dependent on the expression of self-peptide:MHC class I. This feature of the IL-15 complex response may mimic the action of IL-15 during immune responses, in which higher-avidity T cells may compete more effectively for homeostatic cytokines. IL-15 complex holds promise as an immunotherapy, but further study of the synergy between TCR and IL-15R signaling is required to aid in the design of therapeutic protocols.

Abbreviations used in this paper

hIL-15	human IL-15
IL-2 complex	IL-2α/IL-2
peptide/IL-15 complex	SIINFEKL peptide and IL-15 complex
pLN	peripheral lymph node
VSV-OVA	vesicular stomatitis virus expressing OVA

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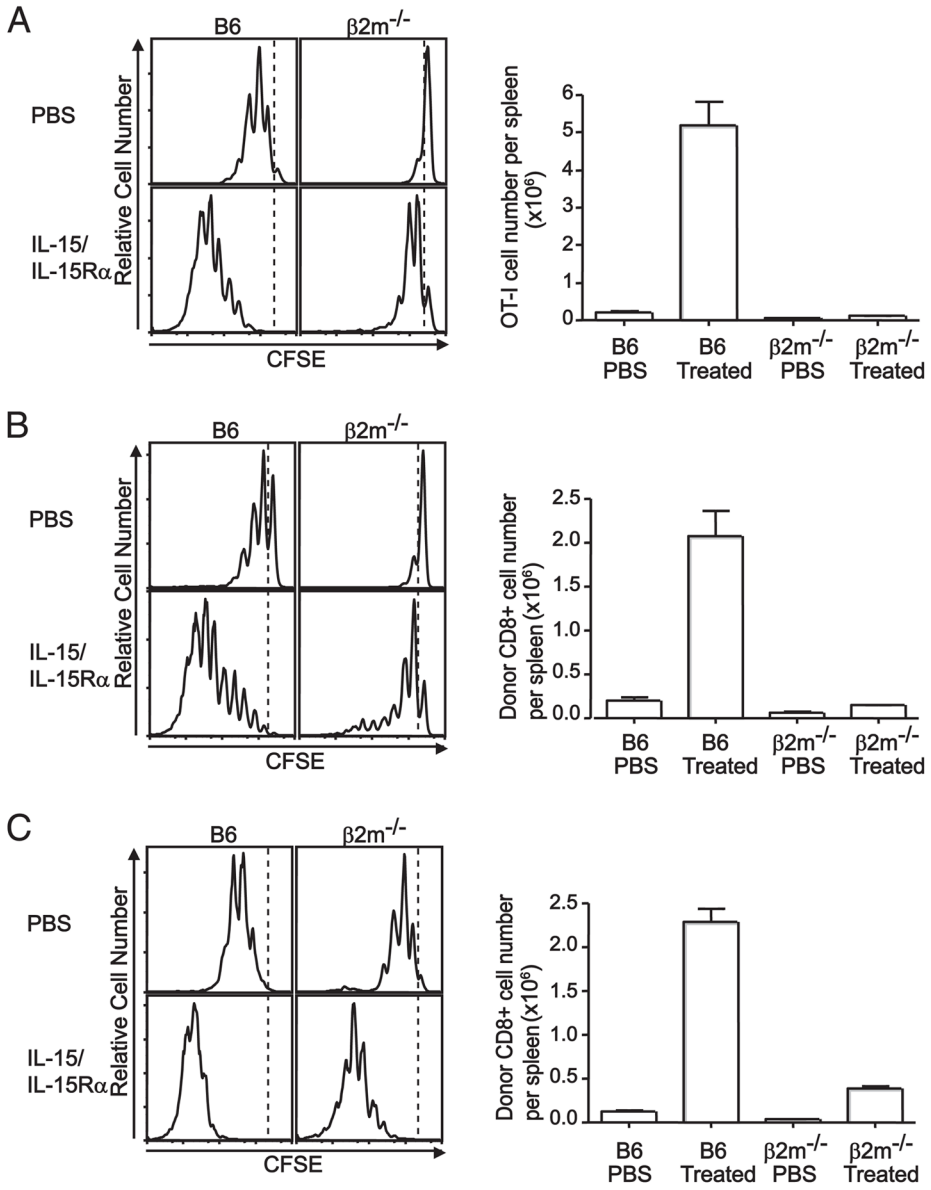

FIGURE 1.

Naive CD8 T cell response to IL-15 complex requires MHC class I. *A*, A total of 5×10^5 CFSE-labeled naive CD45.1 $CD44^{lo}$ OT-I TCR transgenic CD8 T cells was injected i.v. into CD45.2 B6 or $\beta2m^{-/-}$ hosts that were sublethally irradiated (500 rad) 12 h earlier. One day later, mice were injected i.p. with PBS or 2.5 μg hIL-15 + 15 μg mIL-15Rα–Fc. Four days after treatment, donor cell proliferation and accumulation were examined in the spleen. A total of 5×10^5 CFSE-labeled CD45.1 $CD44^{lo}$ polyclonal CD8 T cells (*B*) and 3×10^5 CFSE-labeled Thy1.1 $CD44^{hi}$ polyclonal CD8 T cells (*C*) were coinjected i.v. into CD45.2 Thy1.2 B6 and $\beta2m^{-/-}$ mice that were sublethally irradiated (500 rad) 12 h earlier. One day later, mice were injected i.p. with PBS or 2.5 μg hIL-15 + 15 μg mouse IL-15Rα–Fc. Four days later, donor cell proliferation and accumulation were examined in the spleen. Data are representative of at least two experiments ($n = 3$).

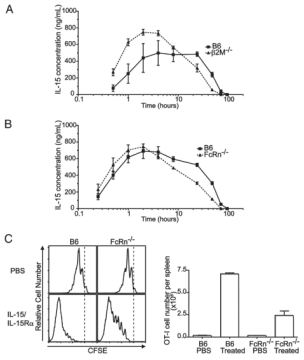

FIGURE 2.

Naive CD8 T cell response to IL-15 complex is partially dependent on FcRn. *A*, B6 and $\beta 2M^{-/-}$ mice were injected i.p. with 2.5 μg hIL-15 + 15 μg mouse IL-15Rα–Fc, and hIL-15 was measured in the blood at various time points after injection (0, 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h) by ELISA. *B*, As in *A*, but using B6 and $FcRn^{-/-}$ mice and evaluating at ~0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h. *C*, A total of 4.0×10^5 CFSE-labeled CD45.1 $CD44^{lo}$ OT-I CD8 T cells were injected i.v. into CD45.2 B6 or $FcRn^{-/-}$ mice sublethally irradiated (500 rad) 12 h earlier. One day later, mice were treated with PBS or 2.5 μg hIL-15 + 15 μg mouse IL-15Rα–Fc. Four days later, donor cell proliferation and accumulation were examined in the spleen. Data are representative of at least two experiments (n = 2–4).

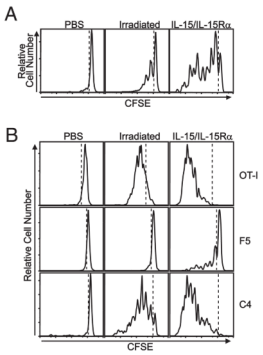

FIGURE 3.

Naive CD8 T cell response to IL-15 complex is heterogeneous. $CD44^{lo}$ polyclonal CD8 T cells (*A*) or OT-I, F5, or C4 CFSE-labeled CD45.1 $CD44^{lo}$ CD8 T cells (*B*) were injected i.v. into irradiated (500 rad) or normal CD45.2 B6 or B10.d2 (for C4) mice. One day later, the normal B6 mice received PBS or 2.5 μg hIL-15 + 15 μg mouse IL-15Rα–Fc i.p. Five days after treatment, donor cell proliferation was examined in the spleen.

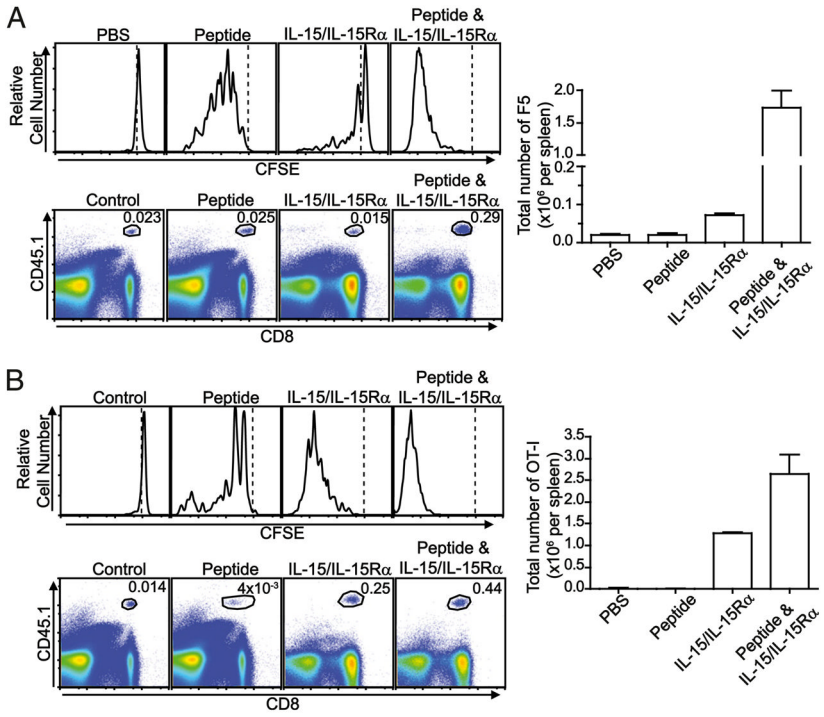

FIGURE 4.

TCR avidity controls the CD8 T cell response to IL-15 complex. F5 (*A*) or OT-I CFSE-labeled CD45.1 CD44^{lo} (*B*) CD8 T cells were injected i.v. into B6 mice. One day later, mice were treated i.p. with PBS, IL-15 complex, or with F5-NP peptide (100 μg) or SIINFEKL (6.25 μg), or with peptide plus IL-15 complex. Five days after treatment, donor cells were examined for proliferation and total cell numbers in the spleen. Data are representative of two similar experiments (n = 3).

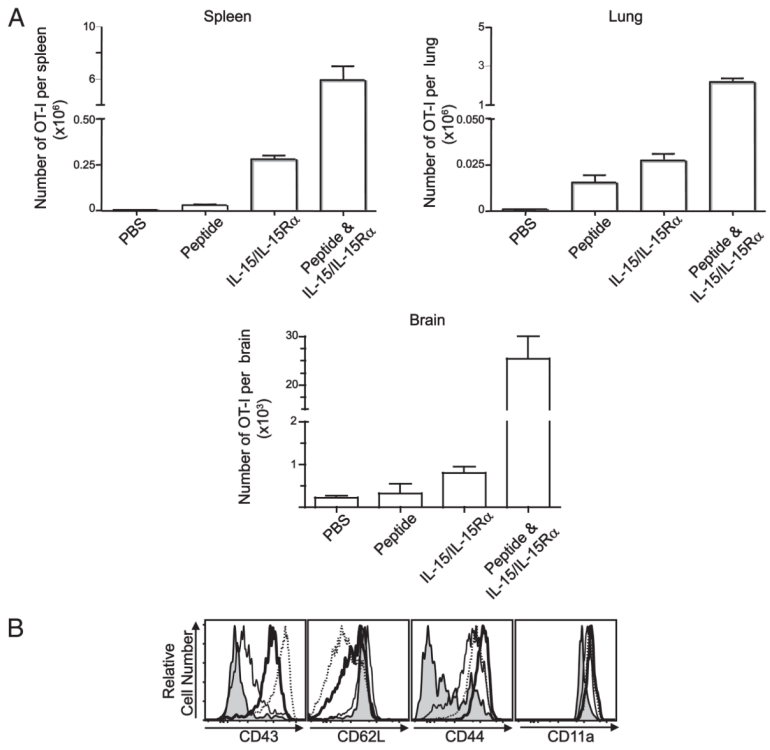

FIGURE 5.

Cognate peptide increases and alters the naive CD8 T cell response to IL-15 complex. *A*, CD45.1 CD44^{lo} OT-I CD8 T cells were i.v. transferred into CD45.2 B6 mice. One day later, mice were treated i.p. with PBS, 100 μg SIINFEKL peptide,2.5 μg hIL-15 + 15 μg IL-15Rα–Fc, or 100 μg SIINFEKL and 2.5 μg IL-15 + 15 μg IL-15Rα–Fc. Donor cell accumulation was examined in the spleen, lungs, and brain. *B*, Donor cells from the spleen were analyzed for expression of the indicated proteins by flow cytometry. Shaded graph = PBS, thin line = IL-15 + IL-15Rα–Fc, thick line = peptide, dashed line = peptide + IL-15 + IL-15Rα–Fc. Data are representative of at least two experiments (n = 3–4).

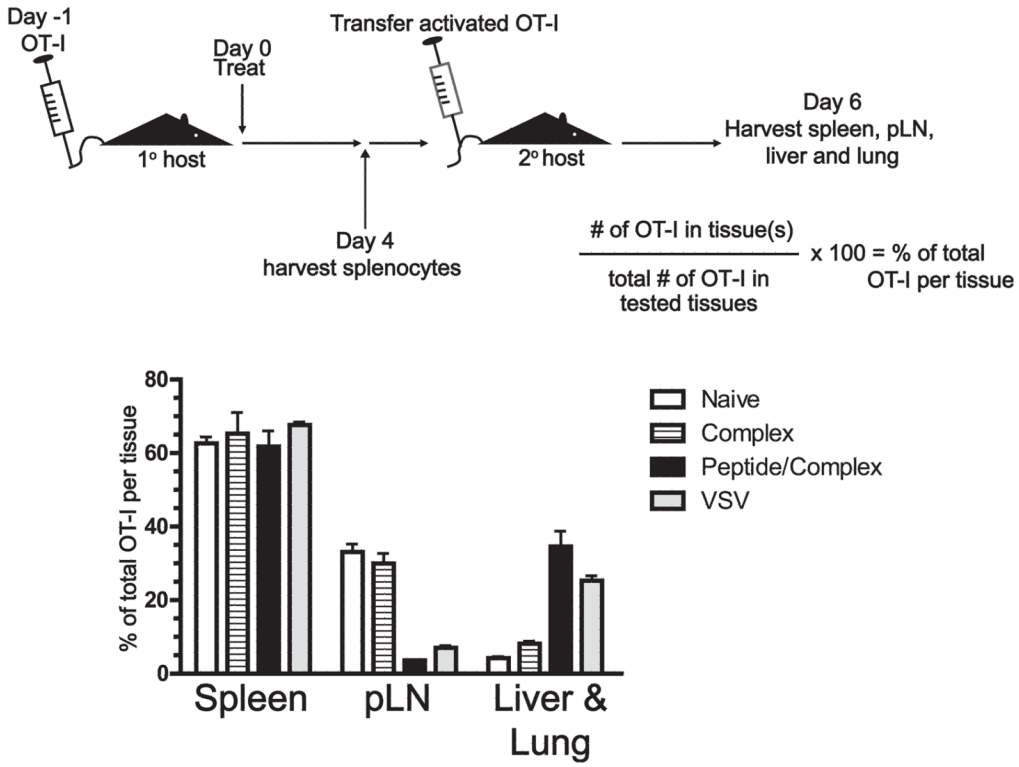

FIGURE 6.

Activation with cognate peptide and IL-15 complex alters the migratory ability of CD8 T cells. A total of 6×10^6 congenic naive OT-I cells were transferred into B6 hosts on day −1 and then were treated i.p. with 2.5 μg IL-15 + 15 μg IL-15Rα–Fc, 100 μg SIINFEKL and 2.5 μg IL-15 + 15 μg IL-15Rα–Fc, or 1×10^5 PFU VSV-OVA on day 0. Four days posttreatment, splenocytes were harvested and enriched for donor OT-I cells. OT-I cells (1×10^6) from each group, including fresh $CD44^{lo}$ naive OT-I cells, were transferred i.v. into secondary untreated B6 hosts. Two days after secondary transfer, mice were sacrificed, and the presence of donor OT-I cells was determined in spleen, pLNs, liver, and lung. Data are depicted as the percentage of cells in a tissue out of four tissues tested and are representative of two experiments ($n = 3$).