DISTINCTIONS IN LYMPHOCYTE RESPONSES TO IL-2 AND IL-15 REFLECT DIFFERENTIAL LIGAND BINDING INTERACTIONS WITH THE IL-2Rβ CHAIN AND SUGGEST DIFFERENTIAL ROLES FOR THE IL-2Rα AND IL-15Rα SUBUNITS

J.L.O. de Jong, N.L. Farner, P.M. Sondel

More interleukin 15 (IL-15) than IL-2 was needed to generate comparable proliferative responses by phytohaemagglutinin (PHA) blasts and Tf-1β cells expressing high affinity and intermediate affinity IL-2 receptor (IL-2R) complexes, respectively. The focus of these experiments was to determine the contribution of the shared IL-2 and IL-15 receptor components to these dose-response differences. Some of this difference can be attributed to the role of the IL-2Rβ chain, in that HuMikβ1, a monoclonal antibody recognizing the IL-2Rβ chain, blocks 92.2 ± 2.5% (mean ± SE) of the IL-2 proliferative response by Tf-1β cells but only inhibits 57.9 ± 3.7% of the IL-15 response, indicating that IL-2 and IL-15 may physically utilize the IL-2Rβ chain differently. Monoclonal antibody 34L, which recognizes IL-2Rβ but does not inhibit IL-2 binding to the IL-2Rβ chain, blocks 35.4 ± 2.3% of IL-15-stimulated proliferation of PHA blasts, while not affecting the IL-2-stimulated proliferation. Finally, although HuMikβ1 does not inhibit IL-2 responses by PHA blasts bearing high affinity IL-2 receptors, HuMikβ1 does block IL-15-stimulated proliferation by these same cells bearing high affinity IL-15 receptors (88.5 ± 1.6% inhibition). This indicates that the role of IL-15Rα in the high affinity IL-15R complex is distinct from that of IL-2Rα in the high affinity IL-2R complex. Overall, these studies show that the physical interactions of the IL-2Rβγc complex with IL-2 are different than the interactions with IL-15.

Interleukin 15 (IL-15) and IL-2 share several functional activities, including the ability to activate proliferation of T cells, B cells and NK cells, as well as lytic activity of NK cells. While most cell types that respond to IL-2 also respond to IL-15, a greater dose of IL-15 is often required to achieve the same level of response. One hypothesis that could account for these quantitative distinctions is that the different dose–response relationships for IL-2 and IL-15 might result from functional differences between the IL-2 and IL-15 molecules, or differences in their physical interaction with their respective receptor complexes.

The IL-2 receptor (IL-2R) complex includes three transmembrane glycoproteins: the 55-kDa IL-2Rα chain, the 70–75-kDa IL-2Rβ chain and the 64-kDa γc chain. The high affinity IL-2R (Kd = 2–50 pM) consists of an αβγc heterotrimeric complex and is expressed on activated T cells. These cells express an excess of IL-2Rα chains which bind IL-2 with low affinity (Kd = 2–20 nM), so that cells expressing all three IL-2R components exhibit both high (αβγc) receptor complexes) and low (IL-2Rα monomers) affinity binding. Cells expressing IL-2Rβ and γc, but not IL-2Rα chains, exhibit intermediate affinity binding (Kd = 0.5–2 nM) to IL-2.

The IL-15R complex shares two subunits in common with the IL-2R complex. The intermediate affinity human IL-15R consists of IL-2Rβ and γc (Kd = 0.27–2.5 nM). Both the IL-2 and IL-15 receptor complexes need both IL-2Rβ and γc to support signal transduction; however, a degree of specificity may be afforded by the contributions of each unique alpha chain, the IL-2Rα and IL-15Rα molecules, respectively. The IL-15Rα chain is a 58–60-kDa protein which shares structural similarity with the IL-2Rα chain, but differs from the IL-2Rα chain in that it is able to bind IL-15 with high affinity (Kd = 10−11 M). Conversely, IL-2Rα alone binds to IL-2 with low affinity (Kd = 10−3 M). The IL-2Rα and IL-15Rα chains do not possess signalling capabilities.
In order to explore more fully the hypothesis that quantitative differences in IL-2 and IL-15 proliferative responses are due to distinct extracellular interactions with their shared receptor components, proliferative responses by phytohaemagglutinin (PHA) blasts expressing high affinity IL-2R complexes, and Tf-1β cells expressing intermediate affinity IL-2R complexes were compared in the presence of antibodies against the IL-2Rα or IL-2Rβ chains. In addition, responses of these cells to F42K, an IL-2 variant which does not bind to the IL-2Rα chain, were compared with responses to IL-15, as both these cytokines use the IL-2Rβ and γc subunits, but not the IL-2Rα subunit.

**RESULTS**

**IL-15 is less potent than IL-2 in stimulating proliferation of cells bearing either high affinity IL-2R or intermediate affinity IL-2R**

Initial studies examined proliferative responses induced by IL-2 or IL-15 of PBMC from healthy donors that were first activated in vitro with PHA. These PHA blasts consisted of a heterogeneous population of lymphocytes which was at least 95% CD3+ as measured by flow cytometry (data not shown), indicating that these cells were predominantly activated T cells expressing high affinity IL-2 receptors. PHA blasts were incubated with increasing concentrations of IL-2, IL-15 or F42K, an IL-2 variant which does not bind to the IL-2Rα subunit. Previous reports have shown that IL-2 and F42K stimulate comparable responses in cells expressing intermediate affinity IL-2 receptors, while F42K binds to and stimulates high affinity IL-2 receptors less effectively than does IL-2. All three cytokines stimulated proliferation of PHA blasts (Fig. 1A), but different concentrations of each cytokine were required to reach a comparable level of [3H]thymidine incorporation. We found that IL-2 stimulated proliferation of PHA blasts most effectively, followed by F42K, and finally IL-15. For IL-2, the effective concentration needed to achieve a 50% maximal response (EC50, mean ± SE) was 60.8 ± 43 pM (n = 4); for F42K the EC50 = 992 ± 263 pM (n = 4), and for IL-15 the EC50 = 6.13 ± 0.54 nM (n = 4) (Table 1). The mean EC50 value for each cytokine was significantly different from each of the other cytokines, with P < 0.0005 in all cases.

To compare IL-2 and IL-15 responses in a cell line which expresses intermediate affinity rather than high affinity IL-2R complexes, the Tf-1β myelomonocytic cell line was used. These cells express γc molecules endogenously and express the IL-2Rβ chain from a retroviral vector. Unlike PHA blasts, Tf-1β cells do not express the IL-2Rα chain, and hence display intermediate affinity binding to IL-2 rather than high affinity binding. When Tf-1β cells were stimulated by IL-2, IL-15 or F42K, the IL-2 and F42K activation induced similar dose response curves, as expected in cells bearing intermediate affinity IL-2R. However, IL-15 stimulated a less potent response than either IL-2 or F42K (Fig. 1B). The EC50 (mean ± SE) was 60.8 ± 43 pM (n = 4) for IL-2, 992 ± 263 pM (n = 4) for F42K, and 6.13 ± 0.54 nM (n = 4) for IL-15 (Table 1).

**TABLE 1. Mean effective concentration of cytokine needed to reach 50% maximal stimulation (EC50) upon stimulation of PHA blasts and Tf-1β cells**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PHA blasts</th>
<th>Tf-1β</th>
</tr>
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<tbody>
<tr>
<td>IL-2</td>
<td>60.8 ± 43 pM (4)</td>
<td>59.9 ± 12.7 pM (8)</td>
</tr>
<tr>
<td>F42K</td>
<td>992 ± 263 pM (4)*</td>
<td>108 ± 7 pM (4)</td>
</tr>
<tr>
<td>IL-15</td>
<td>6.13 ± 0.54 nM (4)*</td>
<td>183 ± 36 pM (8)*</td>
</tr>
</tbody>
</table>

EC50 values are presented as mean ± SE. The number of experiments (n) is given in parentheses. P values were calculated from LSD comparisons and one-way ANOVA.

*Significantly different from IL-2 (P < 0.01).
59.9 ± 12.7 pM (n = 8) for IL-2; for F42K EC\textsubscript{50} = 108 ± 7 pM (n = 4), and for IL-15 EC\textsubscript{50} = 183 ± 36 pM (n = 8) (Table 1). The means of the EC\textsubscript{50} values for IL-2 and F42K were not significantly different (P = 0.18), while IL-2 was greatly different from IL-15 (P = 0.0012). These results contrasted with the data from PHA blasts bearing high affinity IL-2 receptors, in that much greater concentrations of IL-15 and F42K (based on the EC\textsubscript{50} values) were needed to reach half-maximal proliferation of PHA blast cells compared to Tf-1β cells. Hence these data suggest that IL-15 responses may be negatively influenced by the expression of IL-2Rξ. In addition, the fact that F42K is more potent than IL-15 (ie. has a lower EC\textsubscript{50} value than IL-15) for both PHA blasts and Tf-1β cells indicates that the quantitative differences in proliferative responses between IL-2 and IL-15 are not solely due to the contribution of the IL-2Rξ subunit.

**Antibodies against the IL-2Rβ chain inhibit IL-2- and IL-15-stimulated proliferation to different degrees, indicating different roles for IL-2Rξ and IL-15Rξ in their respective extracellular receptor–ligand interactions**

To test whether the quantitative differences between IL-2 and IL-15 proliferative responses might also be influenced by different physical interactions with the IL-2Rβ chain, additional experiments compared the ability of anti-IL-2Rβ antibodies to block proliferative responses to increasing doses of IL-2 or IL-15 in cells bearing high affinity or intermediate affinity IL-2 receptors. Two different monoclonal antibodies were examined: mAb 341 and humanized Mikβ1 (HuMikβ1). HuMikβ1 inhibits IL-2 from binding to the IL-2Rβ chain, thus blocking IL-2 from interacting with the intermediate affinity IL-2R complex.\textsuperscript{2,3} Indeed, IL-2-stimulated proliferation of TF-1β cells is completely abrogated by HuMikβ1 (Fig. 2A). In contrast, HuMikβ1 only inhibits approximately 65% of the response to IL-15 by TF-1β cells (Fig. 2B). One hypothesis to account for this observation is that IL-15 may interact with the IL-2Rβ chain in a different region of the IL-2Rβ molecule which is not blocked by the HuMikβ1 antibody, and is distinct from the epitope on IL-2Rβ bound by IL-2. Alternatively, IL-15 may bind to a separate receptor subunit (such as IL-15Rξ) that is not blocked by HuMikβ1, and this binding may partially compensate for the inhibitory effect of the HuMikβ1 antibody. The second antibody used in these studies against the IL-2Rβ subunit was mAb 341, which does not inhibit IL-2 from binding the IL-2Rβ chain.\textsuperscript{20} As expected, TF-1β proliferation to IL-2 or IL-15 is not affected by the addition of this mAb 341 (Fig. 2).

These blocking studies with HuMikβ1 and mAb 341 were repeated on OKT3 blasts expressing high affinity IL-2R (Fig. 3). OKT3 blasts are a heterogeneous population of activated T cells, and are comparable to PHA blasts in their responses to IL-2 and IL-15 and in their expression of IL-2 receptor components (data not shown). As expected, the anti-IL-2Rξ mAb GL439 inhibited 95% of proliferation mediated via high affinity IL-2 receptors stimulated by low concentrations of IL-2 (Fig. 3A), but did not alter the response to IL-15 (Fig. 3B). Surprisingly, mAb 341 blocked approximately 20% of the response of OKT3 blasts to IL-15, although this antibody does not block IL-2 binding and did not affect the response of OKT3 blasts to IL-2 (Fig. 3). As published previously,\textsuperscript{22} HuMikβ1 did not inhibit IL-2 stimulation of OKT3 blasts. In their report, Tsudo et al. established that although Mikβ1 blocks the binding of IL-2 to the IL-2Rβ chain, the affinity of Mikβ1 for the IL-2Rβ chain (\(K_d = 4\) nM) is sufficiently lower than...
the affinity of IL-2 for the high affinity trimolecular αβγ complex \( (K_2 = 10 \text{ pM}) \), so that this Mikβ1 antibody does not inhibit IL-2-stimulated responses of high affinity receptor bearing cells.\(^{22}\) This observation is confirmed in our studies here, however an important difference is noted in the IL-15 responses. HuMikβ1 did block approximately 80% of the IL-15-stimulated proliferation of these OKT3 blasts, despite the fact that these T cells express high affinity IL-15R, as determined by Scatchard binding analyses.\(^{10-16}\) Therefore, IL-15 appears to utilize its IL-15Rα subunit differently from the way that IL-2 utilizes its IL-2Rα subunit on OKT3 blasts. IL-2Rα combines with the IL-2Rβγ complex to convert intermediate affinity receptors into high affinity IL-2R. IL-15Rα, which alone can bind IL-15 with high affinity, appears not to serve this same type of “affinity converting” function for the βγ complex because the IL-15 response (but not the IL-2 response) on OKT3 blasts can be partially blocked by HuMikβ1. The fact that HuMikβ1 antibody does not completely inhibit the IL-15 response by OKT3 blasts, suggests that either IL-15 interacts with an epitope on the IL2Rβ chain distinct from that of IL-2, or that perhaps the IL-15Rα can overcome a portion of the blocking by HuMikβ1.

**Figure 3.** Anti-IL-2Rβ antibodies (HuMikβ1 and mAb 341) inhibit IL-15-stimulated proliferation of OKT3 blasts.

The responses to IL-2 (A) and IL-15 (B) were analysed in the presence of media alone (○), MOPC21 isotype control antibody (○), mAb 341, a non-inhibitory antibody against IL-2Rβ (▲), anti-IL-2Rα antibody GL439 (△), human serum, as a control (■), and the humanized anti-IL-2Rβ antibody HuMikβ1 (□). The final concentration of each antibody in the well was 50 μg/ml. An appropriate range of cytokine concentrations was selected for each experiment based on the EC50 values for IL-2 and IL-15. HuMikβ1 did not affect IL-2 stimulated proliferation, but suppressed approximately 80% of the IL-15 stimulated proliferation. Anti-IL-2Rα antibody GL439 blocked 95% of IL-2 stimulated proliferation, but did not affect IL-15 stimulation. The GL439 data are difficult to visualize in panel B, as the control data and the data with the GL439 antibody are virtually superimposed. The largest error bar in B at the greatest concentration of IL-15 coincides with the GL439 data point. mAb 341, which does not block IL-2 binding to the IL-2Rβ chain, did not alter IL-2 stimulated proliferation, but did inhibit approximately 20% of IL-15-stimulated proliferation.

**Increasing the concentration of HuMikβ1 anti-IL-2Rβ blocking antibody saturates the inhibitory response, but does not completely abrogate IL-15-stimulated proliferation**

The data illustrated in Figures 2 and 3 were generated using varying concentrations of cytokine with a fixed concentration of blocking antibody (50 μg/ml), and did not result in complete blocking of proliferation under some conditions. In order to verify the reliability of these results, the converse experiment also needed to be performed using a fixed concentration of cytokine and varying dilutions of antibody. The data suggested that IL-15 and IL-2 might bind to the extracellular portion of the IL-2Rβ chain differently, and hence it was important to determine if the IL-15 response could be completely inhibited merely by increasing the concentration of blocking antibody relative to the concentration of cytokine. In order to test this hypothesis, PHA blasts and Tf-1β cells were tested under conditions with titrated concentrations of blocking antibodies and a constant concentration of cytokine, apparently equal to the EC50 value. By increasing the concentration of antibody relative to cytokine, we hoped to show whether the partial inhibition of IL-15-stimulated proliferation could be increased to 100% inhibition.

As indicated by the representative experiment with Tf-1β cells shown in Figure 4, HuMikβ1 antibody inhibits nearly all of the IL-2 stimulated proliferation. Maximal inhibition is reached in the presence of approximately 5 μg/ml of HuMikβ1, as indicated by the plateau in the inhibition curve (Fig. 4A). In five separate experiments, the mean inhibition of IL-2 stimulation was 92.2% ± 2.5%, under these conditions (Table 2). In contrast, the proliferative response to IL-15 by Tf-1β cells is only partially abrogated by HuMikβ1 antibody, and the inhibition curve also plateaus at approximately 5 μg/ml of HuMikβ1 antibody (Fig. 4B). In five separate experiments, the mean inhibition was 57.9% ± 3.7%. These data indicate that increasing the concentration of HuMikβ1 blocking antibody will not result in greater inhibition of IL-15-stimulated proliferation, and therefore suggest
that IL-15 physically interacts differently with the IL-2Rβ chain than IL-2 does.

These same experiments were repeated using PHA blasts and a representative experiment is shown in Figure 5. As expected, anti-IL-2Rα antibody GL439 blocked 93.6% ± 2.7% (n = 7) of the IL-2-stimulated response (Fig. 5A), but did not alter IL-15 stimulated proliferation (inhibition of −3.4% ± 4.4%, n = 7) (Fig. 5B, Table 2). Anti-IL-2Rβ antibody 341, which does not block IL-2 binding to the IL-2Rβ chain, also did not alter IL-2 stimulated proliferation of these PHA blasts (Fig. 5A). However, 341 did significantly inhibit proliferation activated by IL-15 (35.4% ± 2.3%, n = 3), demonstrating again that IL-15 might bind to the IL-2Rβ chain differently than IL-2 does (Fig. 5B).

As expected, HuMikβ1 does not inhibit proliferation of PHA blasts stimulated by low concentrations of IL-2 (Fig. 5A). However, at antibody concentrations greater than 10 μg/ml, we observed a small degree of inhibition (21.9 ± 3.5%, n = 7). In contrast, HuMikβ1 inhibits 88.5% ± 1.6% (n = 7) of IL-15-stimulated responses by PHA blasts (Fig. 5B). This occurs despite expression of high affinity IL-15 binding by these cells, suggesting that the IL-15Rα chain and the IL-2Rα serve distinct roles for ligand binding in their respective high affinity receptor complexes. The results from the experiments in Figure 5 are similar for either PHA blasts or KIT225 cells, an IL-2 dependent human T cell line (data not shown).

**Antibody against IL-2 does not inhibit IL-15 stimulated responses by PHA blasts**

Anti-IL-15 antibody M111 and polyclonal anti-IL-2 rabbit anti serum were used to confirm that the responses documented here using PHA blasts were truly mediated by IL-15, and not due to the induction of IL-2 as a secondary cytokine. Proliferation of PHA blasts in response to IL-2 is blocked only by the anti-IL-2 antisera, and not the anti-IL-15 antibody, M111 (Fig. 6). Likewise, IL-15 stimulated proliferation is inhibited by anti-IL-15, and not by anti-IL-2 antibody. Similar results have been documented using Tf-1β cells (Farner et al., submitted).

**DISCUSSION**

Although IL-2 and IL-15 share many functional responses, we noted that often a greater quantity of IL-15 was required to stimulate the same level response as IL-2. While this observation might be explained merely by a lower specific activity of the IL-15 reagent compared to IL-2, this possibility seemed unlikely in that cytotoxicity dose–response curves for IL-2 and IL-15 using these same reagent stocks were not different from each other. A more intriguing hypothesis to explain these proliferation dose–response differences is that IL-2 and IL-15 may bind to extracellular portions of shared receptor components differently. In order to establish these functional distinctions fully, cells bearing high affinity IL-2R (that is, PHA and OKT3 blasts), and cells bearing intermediate affinity IL-2R (Tf-1β cells) were subjected to proliferative assays containing IL-2, IL-15 or F42K. Regardless of whether the responding cells expressed high affinity or intermediate affinity receptors, the EC₅₀
value for IL-15 was always significantly higher than that of IL-2 (Table 1). This result indicated that more IL-15 than IL-2 is required to activate half-maximal proliferation of each of these cell types, independent of IL-2Rα expression.

The IL-2 variant, F42K, allowed us to clarify further the influence of IL-2Rα expression in those assays involving cells with high affinity IL-2R, as F42K does not bind to this subunit. If the quantitative differences between IL-2 and IL-15 responses were due to receptor differences, then an obvious candidate for mediating these differences would be the IL-2Rα chain since IL-15 does not utilize this subunit. If the IL-2Rα chain were the sole factor accounting for the differential proliferative responses to IL-2 and IL-15, then the results with F42K (which like IL-15 does not utilize the IL-2Rα molecule) should mimic those with IL-15. However, the mean EC50 for IL-15 was significantly higher than that of F42K for PHA blasts and Tf-1β cells, \( (P = 0.0004 \text{ and } 0.05, \text{ respectively}) \), indicating that the IL-2Rα chain was not the only determinant influencing the quantitatively distinct proliferative responses induced by IL-2 and IL-15. This is consistent with previously published data using the inhibitory anti-IL-2Rα mAb, GL439. Inclusion of mAb GL439 had no influence on IL-15-stimulated proliferation, but inhibited the IL-2 response, resulting in a shift of the IL-2 dose–response curve to the right, although not as far to the right as the IL-15 dose–response curve.\(^{16}\) This again suggested that other determinants, besides the IL-2Rα subunits, were affecting this quantitative response differential.

Previously published molecular data suggest that IL-2Rβ and γc interact more strongly with IL-2 than with IL-15.\(^{16}\) Co-immunoprecipitation experiments have proven that the interactions between IL-2, IL-2Rβ and γc are sufficiently strong to allow co-precipitation of this βγc complex from intermediate affinity receptor bearing YT cells when incubated with IL-2.\(^{20}\) However, IL-15 interacts more weakly with the βγc complex so that immunoprecipitates with anti-IL-2Rβ antibodies do not precipitate γc along with β in the presence of IL-15.\(^{16}\) Therefore, IL-15 may not interact as strongly with the βγc complex as IL-2 does, potentially resulting in the functional differences reported here. Further binding assays to determine association and dissociation constants for IL-2 and IL-15 need to be performed in order to fully clarify these differences. Taken together with the proliferative results using F42K and anti-IL-2Rα antibodies on both high affinity and intermediate affinity receptor bearing cells, it appears that IL-15 may not interact as strongly with the βγc complex as does IL-2, independent of the influence of the IL-2Rα molecule. This weaker extracellular interaction may have functional significance which impacts on proliferative response.

Additional experiments examined the ability of antibodies directed against the IL-2Rβ chain to affect IL-2 and IL-15-stimulated proliferation of cells bearing high or intermediate affinity IL-2R. The anti-IL-2Rβ antibody, HuMikβ1, did not influence the response of PHA blasts to IL-2, but did block proliferation of Tf-1β cells in response to IL-2. In contrast, IL-15-stimulated proliferation of both PHA blasts and Tf-1β cells was blocked by HuMikβ1, although not completely. In addition, the mAb 341, which does not inhibit the response to IL-2, had no effect on IL-15 stimulated proliferation of Tf-1β cells, but was able to inhibit a small portion of the proliferation of PHA blasts stimulated by IL-15. Not only do these data indicate distinctions between binding of the IL-2Rβ chain by IL-15 and IL-2, but they also suggest that the IL-15Rα chain functions differently than its IL-2Rα counterpart within their respective high affinity IL-15

### Table 2. Statistical analysis of inhibition of proliferation by anti-receptor antibodies

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cytokine</th>
<th>Antibody</th>
<th>% Inhibition relative to control</th>
<th>P value when compared to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA blasts</td>
<td>IL-2</td>
<td>GL439</td>
<td>93.6 ± 2.7% (n = 7)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>341</td>
<td>8.1 ± 9.2% (n = 3)</td>
<td>0.54</td>
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<tr>
<td></td>
<td></td>
<td>HuMikβ1</td>
<td>21.9 ± 3.5% (n = 7)</td>
<td>0.001*</td>
</tr>
<tr>
<td>PHA blasts</td>
<td>IL-15</td>
<td>GL439</td>
<td>−3.4 ± 4.4% (n = 7)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>341</td>
<td>35.4 ± 2.3% (n = 3)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HuMikβ1</td>
<td>88.5 ± 1.6% (n = 7)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Tf-1β cells</td>
<td>IL-2</td>
<td>GL439</td>
<td>−1.1 ± 7.0% (n = 5)</td>
<td>0.14</td>
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<td></td>
<td></td>
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<td>4.6 ± 2.7% (n = 5)</td>
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<td></td>
<td></td>
<td>HuMikβ1</td>
<td>92.2 ± 2.5% (n = 5)</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Tf-1β cells</td>
<td>IL-15</td>
<td>GL439</td>
<td>−3.7 ± 3.3% (n = 5)</td>
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<td></td>
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<td>341</td>
<td>1.9 ± 4.4% (n = 5)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>HuMikβ1</td>
<td>57.9 ± 3.7% (n = 5)</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

Proliferative responses to IL-2 and IL-5 were measured in media alone (control values), or in the presence of antibodies directed against the IL-2Rα chain (GL439), or the IL-2Rβ chain (341 and HuMikβ1). The values obtained in the presence of antibody are expressed as percentage inhibition and are presented as mean ± SE. The number of experiments (n) is given in parentheses. P values were calculated from LSD comparisons and one-way ANOVA.

*Significantly different from isotype control (\( P < 0.001 \)).
These blocking data are also consistent with the hypothesis that different populations of receptors which interact with IL-2 and IL-15 may be simultaneously present on the same cell surface. As has been shown previously, IL-2 receptors exist in two forms on activated T cells: an abundance of low affinity receptors (IL-2Rα alone) and a few high affinity receptors (trimolecular αβγc complexes). All the IL-2 receptors capable of signalling in response to IL-2 on these IL-2Rα complexing cells have high affinity for IL-2 because the IL-2Rα chain is expressed in excess.6

In contrast, if the IL-15Rα chain were the limiting subunit required for high affinity binding, then two populations of IL-15 receptors might exist simultaneously on the cell surface which are each capable of signal transduction: high affinity IL-15Rαβγc complexes, and intermediate affinity βγc complexes (shown schematically on Tc1β cells in Fig. 7). While IL-15Rα alone is capable of binding IL-15 with high affinity, it does not appear to have signalling capabilities.10 If these two populations of IL-15 receptors were present on a cell, then HuMikβ1 would presumably block the intermediate affinity IL-15Rαβγc complexes, but not the high affinity IL-15RαR and thereby would never be able to block 100% of IL-15-stimulated proliferation of any cell type expressing the IL-15Rα chain, including OKT3 blasts, PHA blasts, and Tc1β cells, even at saturating concentrations of antibody, as the data indicate in Figures 4 and 5. In a heterogeneous population of cells such as OKT3 blasts or PHA blasts, we cannot distinguish whether these two types of IL-15

and IL-2 receptor complexes. Intermediate affinity IL-2R complexes (βγc) are blocked by HuMikβ1 antibody, while high affinity IL-2R complexes (αβγc) are not. The IL-2Rα chain converts the intermediate affinity βγc complex to the high affinity IL-2R, and thereby is not blocked by HuMikβ1 antibody.2 Since IL-15 stimulation is blocked by HuMikβ1 on cells expressing high affinity IL-15 receptors, we conclude that the IL-15Rα is not serving the same purpose on the cell surface as IL-2Rα does in its high affinity receptor.

PHA blasts were stimulated with 20 pM IL-2 (A) or 4 nM IL-15 (B), and incubated with increasing concentrations of various antibodies. Puriﬁed MOPC21 antibody (○) was the isotype control for anti-IL-2Rα antibody GL439 (●). Human serum (▲) served as the isotype control for the humanized anti-IL-2Rβ antibody HuMikβ1 (▲). MOPC21 ascs (■) was the isotype control for mAb 341 ascs, a non-inhibitory anti-IL-2Rβ antibody (□). HuMikβ1 inhibited 21.9% ± 3.3 (n = 7) of IL-2-stimulated proliferation only at the highest concentrations of antibody. However, HuMikβ1 blocked 88.5% ± 1.6 (n = 7, P < 0.0001) of IL-15 stimulated proliferation. Anti-IL-2Rα antibody GL439 blocked 93.6% ± 2.7 (n = 7, P < 0.0001) of IL-2 stimulated proliferation, but did not affect IL-15 stimulation. mAb341, which does not block IL-2 binding to the IL-2Rβ chain, did not alter IL-2 stimulated proliferation, but did inhibit 35.4% ± 2.3 (n = 3, P < 0.0001) of IL-15-stimulated proliferation. Statistical analyses were performed using one-way ANOVA of the data points at 7.5 μg/ml of antibody. P values were determined from least signiﬁcant difference (LSD) comparisons with controls.

PHAG blasts were incubated with IL-2 or IL-15 and 10 μg/ml of anti-IL-2 (polyclonal rabbit anti-IL-2 antibody) or anti-IL-15 antibody mAb M111.

Figure 5. Anti-IL-2Rβ antibody 341, partially inhibits IL-15-stimulated proliferation of PHA blasts.

Figure 6. Proliferation of PHA blasts stimulated by IL-15 is blocked by anti-IL-15 antibody but not anti-IL-2 antibody.
IL-2 and IL-15 receptors on PHA blasts are depicted in the lower panel. Since PHA blasts are a heterogeneous population, we cannot determine whether these receptors are all expressed by the same cell, or by different subpopulations of cells. High affinity IL-2 receptors consist of the IL-2Rαβγ complex, can transduce a signal (indicated by the dark “lightning” arrow), and are blocked by anti-IL-2Rα antibody, GL439. These IL-2Rαβγ complexes are not blocked by HuMikβ1. However, if IL-15 interacts with the IL-2Rαβγ complex, it may serve as an intermediate affinity IL-15R, since IL-15 does not utilize the IL-2Rα chain. Hence IL-15 stimulation of this receptor complex, might be blocked by HuMikβ1. The IL-15Rα chain can bind IL-15 with high affinity all alone, however, this is not believed to be a signal-transducing receptor. If IL-15Rα were to interact with the IL-2Rαβγ complex, then this four-part complex would be expected to bind IL-15 with high affinity and also transduce a signal to the cell. This high affinity IL-15 receptor might not be blocked by HuMikβ1. However, this receptor may be inhibited by mAb 341, as suggested by the blocking data from the experiment presented in Figure 4.

The upper panel depicts IL-2 and IL-15 receptors on the surface of Tf-1β cells. This is a homogeneous, cloned cell line, and presumably all cells express the same receptor subunits. The intermediate affinity IL-15 and IL-2 receptors (βγ) would be blocked by HuMikβ1, consistent with the abrogation of IL-2 stimulation seen in Figure 4A. However, the high affinity IL-15 receptors (IL-15Rαβγ) on these same cells may not be blocked by HuMikβ1. Therefore, even by increasing the HuMikβ1 concentration to a saturating level, one could inhibit some of the IL-15 stimulation, but never completely inhibit IL-15 stimulation of these Tf-1β cells, as observed in Figure 4B. The expression of IL-15Rα on the surface of these cells is postulated, based upon high affinity IL-15 binding observed with Scatchard analysis.

Diagram is not drawn to scale.
receptor complexes are expressed on the same cell, or are expressed on two different subpopulations of cells. However, with a cloned cell line, such as Tf-1β cells, one would expect that all cells express similar receptor components, so two different types of IL-15 receptors capable of signal transduction might be present on these cells.

We should note that surface expression of IL-15Rα on Tf-1β cells, OKT3 blasts and PHA blasts has been postulated based upon Scatchard binding data and IL-15Rα mRNA expression. Surface expression of IL-15Rα protein has been identified only by FACS staining with tagged IL-15, and by cross-linking of radiolabeled tagged IL-15 with subsequent resolution on SDS-PAGE gels. These methods are relatively insensitive however, and do not allow quantitation of IL-15Rα protein levels in comparison to IL-2Rα. At present, no anti-IL-15Rα antibody exists with sufficient affinity to allow quantitative detection of surface IL-15Rα.

The hypothesis proposed above still does not explain why mAb 341, an antibody directed against the IL-2Rβ chain that does not inhibit the response to IL-2, is able to partially block IL-15-stimulated proliferation of cells bearing high affinity IL-2R complexes (PHA blasts) but not cells bearing intermediate affinity IL-2R complexes. Perhaps the presence of the IL-2Rα inhibits the ability of IL-15Rα molecules to interact with and signal through the shared βγ chain signaling complex. It is possible that IL-15 could still stimulate its intermediate affinity βγ chain receptor, even if the IL-2Rα chain were also associated with that same βγ chain complex (Fig. 7). Such an association with the IL-2Rα chain might alter the conformation of the IL-2Rβ chain so that inhibition of IL-15 stimulation by mAb 341 is facilitated. Since IL-2 stimulation is not affected by the addition of mAb 341, this again supports the idea that IL-15 binds the IL-2Rβ chain differently than IL-2 does. The blocking data with mAb 341 are consistent with this hypothesis. Approximately 20% of the IL-15-stimulated response was inhibited by mAb 341, compatible with the idea that only a minority (~20%) of IL-15 receptors on PHA blasts contain the IL-15Rα molecule and would be affected by IL-2Rα (the IL-2Rα + IL-15Rα + βγ chain complex; second from the right in the lower panel of Fig. 7), while the remainder are intermediate affinity IL-15R (i.e. IL-2Rβγ chain) complexes, not influenced in their IL-15 response by the presence of IL-2Rα (far right, lower panel of Fig. 7). Further testing of these hypotheses will require a sensitive method for directly detecting and quantitating IL-15Rα protein on the surface of these cells.

Taken together, the data presented here indicate that quantitative distinctions do exist in proliferative responses stimulated by IL-2 and IL-15, and these may be due to different physical binding interactions with the IL-2Rβ chain. These data support the conclusion that IL-2Rα maintains a different role than that of the IL-15Rα chain in their respective high affinity receptor complexes. In addition, these results support the possibility that two different types of signal transducing IL-15 receptors may be present simultaneously on the surface of a cell, such as a PHA blast. Further studies are warranted to determine the biological significance of these physical differences between IL-2 and IL-15.

**MATERIALS AND METHODS**

**Cell lines**

TF-1 cells were established from a patient with erythroleukemia, and require GM-CSF or IL-3 for growth in vitro. TF-1β cells were generated by infecting the TF-1 human myelomonocytic leukaemia cell line with a retroviral vector containing the IL-2Rβ gene. TF-1β cells were maintained in RPMI complete medium consisting of RPMI 1640 (Bio Whittaker, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT), 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, and 25 mM HEPES (pH 7.4). TF-1β cultures were also supplemented with 5 ng/ml GM-CSF or 50–100 U/ml IL-2. Cells were maintained by culturing at 1–10 × 10^6 cells/ml, and splitting cultures every 2–3 days.

**Generation of blast cell populations**

Fresh peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by centrifugation over a Ficoll-Hypaque density gradient. All volunteer donors signed informed consent documents approved by the UW Committee for the Protection of Human Subjects. OKT3 blasts were established by stimulating fresh PBMC for 4 days with surface immobilized OKT3 (anti-CD3) antibody (Ortho Diagnostics, Raritan, NJ) in RPMI complete medium with 10% human serum substituted for FBS, as described previously. PHA blasts were established by culturing fresh PBMC in RPMI complete medium with 1 µg/ml of purified phytohaemagglutinin (PHA; Burroughs Wellcome Co., Greenville, NC), and 10% human serum (Pel-Freeze, Brown Deer, WI) Substituted for FBS. PHA blasts were cultured for 4 days in PHA. For some experiments these were subsequently cultured in complete medium with 100 U/ml IL-2 for 3–7 days. These treatments to generate OKT3 blasts and PHA blasts resulted in populations consisting predominantly of T cells (>95% CD3+ by flow cytometry, data not shown). By Scatchard binding analysis PHA blasts and OKT3 blasts express high affinity IL-2 receptors and high affinity IL-15 receptors.

**Cytokines**

Recombinant human IL-2 with a specific activity of 1.5 × 10^9 IU/mg was provided by Hoffman La Roche (Nutley, NJ) via the Biological Response Modifiers Program of the National Cancer Institute (Frederick, MD). Each vial of lyophilized powder contained 1 × 10^8 IU of IL-2, 5 mg...
mannitol and 25 mg human albumin. Recombinant human IL-15 was provided by Immunex Research and Development Corporation (Seattle, WA). All IL-15 stock concentrations were determined at Immunex by amino acid analysis using a Beckman 6300 amino acid analyser. Several different lots of IL-15 were utilized for these studies, each stimulating comparable results. F42K is an IL-2 variant which does not bind the IL-2Rz chain due to a point mutation which substitutes lysine for phenylalanine at the 42nd amino acid residue18,19 and was generously provided by Dr Grace Ju of Hoffman La Roche (Nutley, NJ). The concentration of the stock F42K was determined by dividing the OD at 280 nm by the extinction coefficient (0.665, determined experimentally for IL-2).

**Antibodies**

The murine monoclonal antibody (mAb) 341 (IgG1), provided by Dr R. Robb (Oncotherapeutics Inc.), reacts with the IL-2Rβ chain, but does not block binding of IL-2.20 Both ascites and purified preparations of mAb 341 were utilized. Murine monoclonal antibody GLA39 (IgG1), also provided by Dr R Robb, recognizes IL-2Rz and inhibits IL-2 binding to high affinity receptors.21 Murine antibody MOPC21 (IgG1) was used as an isotype control in both purified and ascites preparations (Sigma, St. Louis, MO). The humanized form of the mouse monoclonal antibody Mikβ1 (HuMikβ1) is directed against the IL-2Rβ chain and blocks IL-2 binding to intermediate affinity IL-2 receptors.22,23 HuMikβ1 was generously provided by Dr J. Hakimi (Hoffman La Roche, Nutley, NJ). Human serum (Pel-Freeze, Brown Deer, WI) was used as the control for HuMikβ1. M111 is a murine anti-human IL-15 antibody kindly provided by M. Larson-Marlowe at Genzyme Corporation (Cambridge, MA). Polyclonal rabbit sera against IL-2 was also provided by Dr R. Robb (Oncotherapeutics Inc.).

**In vitro proliferative assay**

Responder cells (1 x 10⁴) were added to triplicate wells of 96-well microtitre plates in a final volume of 200 µl of RPMI 1640 complete medium and increasing concentrations of IL-2, IL-15, or F42K in the presence or absence of monoclonal antibody at a final concentration of 50 µg/ml, unless otherwise noted. Cells were incubated for 48 h at 37°C with 5% CO₂, followed by 12–16 h of pulse labelling with 1 µCi of [³H]thymidine per well. Cultures were harvested onto glass fibre filters using a 96-well Packard FilterMate cell harvester (Packard Instrument Company, Meriden, CT). Dry filters were then counted for 4 min by gas ionization detection with 4 µCi of ²Hthymidine per well. Cultures were then counted for 4 min by gas ionization detection with 85!well Packard FilterMate cell harvester (Packard Instrument Company, Meriden, CT). Dry glass filter samples were then counted for 4 min by gas ionization detection with a 75!well Packard Instrument Company, Meriden, CT. Values are reported as total counts measured in 4 min. EC₅₀ values (effective concentration of cytokine necessary to achieve 50% of maximal stimulation) were calculated using the ALLFIT program, provided by J. Rossio at the National Cancer Institute, Frederick, MD. Blocked one-way analysis of variance (1-way ANOVA) was performed by Dr M. Lindstrom from the Department of Biostatistics at the University of Wisconsin, Madison. Multiple stimuli within each experimental group were further compared using the least significant difference (LSD) method.

**Acknowledgements**

The authors wish to thank Dr. M. Lindstrom for expert statistical analyses and consultation, as well as Drs R. Robb and J. Hakimi for kindly providing antibodies. Finally, we thank Dr M. Widmer of Immunex Research and Development Corporation for generously providing IL-15, and for expert opinions and advice. Supported by ACS-IM-678M and NIH-RO1-CA57778-04.

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