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Regulation of Lymphoid Homeostasis by IL-2 Receptor Signals In Vivo

Danny T. M. Leung, Samantha Morefield, and Dennis M. Willerford

High-affinity IL-2R signals are required for peripheral lymphoid homeostasis in vivo. We found that CD25 was required for regulation of peripheral T cells in mice bearing either the DO11.10 MHC class II-restricted TCR transgene or an Iaβ-null mutation, suggesting that MHC class I- and class II-dependent T cell subsets are regulated independently by IL-2R signals. In contrast, deregulation of serum IgG1 levels in CD25−/− mice was dependent on CD4+ T cells. T cell expansion in DO11.10 CD25−/− mice was not preferential for cells escaping allelic exclusion by the TCR transgene, but was suppressed by a Rag-2-null mutation. Together, these findings suggest that endogenous TCR are required to trigger T cell expansion, but that CD25 regulates T cells activated by low-specificity signals. Expansion of DO11.10 T cells in response to cognate Ag was modestly reduced in CD25−/− mice. These data indicate that the regulatory role of high-affinity IL-2R signals extends beyond the control of Ag-specific responses and suggest a role for these signals in control of bystander T cell activation. The Journal of Immunology, 2000, 164: 3527–3534.

Regulation of Ag responses requires extensive communication among the cellular constituents of the immune system, involving both direct cell-cell interactions and signals mediated by an extensive array of soluble cytokines. Of these, IL-2 is a prototype for T cell responses (1). The IL-2R includes two signal transducing subunits, IL-2Rβ (CD122), which is also shared with the IL-15R, and γc, which is shared among receptors for IL-4, -7, -9, -13, and -15 (2–9). The IL-2Rβ and γc chains are expressed on resting T cells and together can bind IL-2 with low affinity. In T cells activated by TCR ligation or nonspecific stimuli, such as IL-1 or TNF-α, expression of the IL-2Rγc chain (CD25) is induced (9–11). Although this subunit has no known signaling role, it confers the ability to bind IL-2 with high affinity, thus regulating cellular sensitivity to this cytokine (1, 9, 10, 12).

IL-2R signals are generally understood to affect T cell fate after antigenic encounter. IL-2 is a potent growth factor for T cells in vitro, in large part due to effects on promoting the transition from the G1-S phases of the cell cycle following TCR stimulation (1, 13–16). In the setting of appropriate costimulation, TCR ligation also leads to IL-2 secretion, predominantly by the CD4+ T cell subset. Thus, under commonly used culture conditions, T cell proliferation is driven by an autocrine/paracrine hormonal loop (9, 17). These and other observations engendered the view that IL-2 played a key role in amplifying immune responses by mediating clonal expansion of Ag-reactive T cells. This view has become more complicated in recent years, based on the finding that the effects of IL-2 on cell cycle progression can confer sensitivity to cell death induced by further TCR stimulation (18).

The role of IL-2R signals in regulating the immune system has been explored using mice either deficient in IL-2 or lacking the ability to form high-affinity IL-2 receptors through targeted deletion of CD25 (19, 20). Both types of mice exhibit expansion of peripheral lymphoid tissues, hyperscetration of T cell-dependent Ig subclasses, and susceptibility to autoimmune disorders, demonstrating that IL-2R signals are required for tissue homeostasis and self-tolerance (19–22). In both strains, defects in peripheral T cell deletion in response to superantigens has been reported, suggesting that IL-2R signals may be required for efficient activation-induced T cell death in vivo (20, 23). We and others have hypothesized that the lymphoid expansion and autoimmunity observed in mice with defective IL-2R signals is driven by a diverse array of environmental Ags, for which T cell activation is uncoupled from IL-2R-dependent deletion. In this paper, we have examined requirements for CD25 in the homeostatic regulation of several immune system components, including CD4+ and CD8+ T cell subsets, B cells, and CD4+ T cell populations with progressively restricted sets of specificities. The cell-autonomous regulation of T cell Ag responses by CD25 was also examined in the context of a normal lymphoid compartment. These studies indicate a limited role for CD25 in regulating Ag-induced proliferation and cell death in vivo, and suggest that homeostasis is achieved indirectly, possibly through regulation of bystander responses.

Materials and Methods

Mice

CD25−/− mice on a mixed 129/C57BL/6 background (20) were crossed with DO11.10 TCR-transgenic mice (provided by Dr. Abul Abbas, Brigham and Women’s Hospital, Boston, MA) on a BALB/c background (24). Four to five backcrosses with the DO11.10 strain were performed, with offspring selected following flow cytometric analysis for H-2Kβ vs H-2Kα expression (Abs from Pharmingen, San Diego, CA) to ensure homozygosity for the H-2Kβ haplotype. CD25 was genotyped using PCR with the following primers: 5’-GATGAGTGTTTCAACTCAGGTT-3’; 5'-CTTGTTAGGAGGCGCTTTGAAAT-3'; and 5’-TCTCTGCGAGAGAAAGTATCCATCAT-3’. These are specific, respectively, for sequences 5’ of the

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induced mutation, for the deleted segment, and for the inserted neomycin resistance gene (20). The DO11.10 phenotype was determined by staining peripheral blood cells using the KJ1-26 Ab (24). Rag-2 \(^{-/-}\) mice were obtained from Dr. F. W. Alt (Children’s Hospital, Boston, MA). After interbreeding with DO11.10 CD25 \(^{-/-}\) mice, animals were backcrossed to achieve homozygosity of H-2\(^b\), Ia\(^b\) mice (25) were crossed with CD25-mutant mice. Double heterozygotes were intercrossed, and Ia\(^b\) mice were selected based on flow cytometric analysis of peripheral blood for CD4\(^+\) cells. All mice were housed in microisolator cages under pathogen-free conditions.

Flow cytometric analysis

Single-cell suspensions of lymphoid organs were prepared and incubated with the indicated Abs as described previously (20). mAbs conjugated to FITC, PE, biotin, or APC were obtained from PharMingen, except KJ1-26 (hybridoma cells provided by Dr. Abul Abbas), which was prepared in our laboratory and conjugated to FITC or biotin using standard procedures. Secondary staining of biotinylated Abs used streptavidin-CyChrome. For analysis of DO11.10 \(^+\) cells transferred into BALB/c mice, cell suspensions were preincubated with anti-CD16/CD32 (PharMingen) before staining with fluorochrome-conjugated Abs. For analysis of apoptosis, lymph node cells were incubated with FITC-conjugated KJ1-26 and propidium iodide (125 \(\mu\)g/ml) on ice for 30 min. Live and apoptotic KJ1-26 \(^+\) cells were identified using forward scatter vs propidium iodide fluorescence. Stained cell preparations were analyzed using a FACScalibur instrument equipped with dual lasers and CellQuest software (Becton Dickinson, San Jose, CA). Purities of 94–96% as determined by flow cytometry were obtained from the isolation procedure.

Quantitative Southern blot analysis

CD4\(^+\) T cells were purified by incubating cell suspensions with biotin-conjugated Abs to B220 and CD8, followed by streptavidin-conjugated beads and passage over magnetic separation columns (Miltenyi Biotec, Auburn, CA). Purities of 94–96% as determined by flow cytometry were achieved for the isolated cells. Quantitative Southern blot analysis was performed as described previously (26) using a BandH digest of genomic DNA. Germline TCR\(^b\) alleles were detected using a probe encompassing the J\(\beta\)1 segment and lane loading controlled with a probe for the Bcl-2 family protein A1 (27). Band intensity was quantified using a phosphor imager (Bio-Rad, Hercules, CA), and TCR\(^b\) rearrangement was calculated as follows: (density of germline TCR\(^b\) band)/(density of A1 band) \(\times\) (density of kidney germline TCR\(^b\) band)/(density of kidney A1 band).

Immunization of mice

Mice were injected s.c. with synthetic OVA\(_{323-339}\) peptide (600 \(\mu\)g) at two sites over the upper backs on days 0 and 1. On the day of harvest, brachial, axillary, inguinal, and mesenteric lymph node cells of individual mice were dissected and analyzed by flow cytometry. Adoptive transfer of DO11.10 T cells was performed as described elsewhere (28). Lymph node cells were pooled from either DO11.10 CD25 \(^{-/-}\) or DO11.10 CD25 \(^{-/-}\) mice and CD4\(^+\) KJ1-26 \(^+\) cells were quantified using flow cytometry. After normalization for equivalent numbers of these cells, recipient BALB/c mice were injected i.v. with \(-5\times10^8\) lymph node cells via the tail vein. Sixteen hours after transfer (designated as day 0), mice were s.c. challenged with 600 \(\mu\)g of OVA\(_{323-339}\) peptide at two sites over the backs. Lymph node cells were harvested on the indicated days for analysis.

Results

Homeostatic regulation of peripheral lymphoid tissue by CD25

Mice lacking CD25 are unable to regulate the size of the peripheral lymphoid compartment, which expands 5–10-fold by 6 wk of age (20). We sought to investigate how high-affinity IL-2R signals controlled the size of peripheral lymphoid tissues through regulation of the CD4\(^+\) T cell subset before and after Ag administration. The CD25 mutation was bred along with the class II MHC-restricted DO11.10 TCR transgene, which is specific for OVA\(_{323-339}\) peptide (24). Mice were backcrossed to the DO11.10 (BALB/c) background for four to five generations, and homozygosity for H-2\(^d\) was verified. In the studies reported here, CD25 \(^{-/-}\) mice were compared with CD25 \(^{-/-}\) littersmates, which separate analyses have indicated are functionally and phenotypically equivalent to CD25 \(^{-/-}\) mice. Thymus development was assessed using flow cytometry for CD4, CD8, and CD25 expression. Comparison of

![FIGURE 1. Regulation of peripheral lymph node size by CD25. Cell counts in peripheral lymph node from CD25 \(^{-/-}\) (open bars) and CD25 \(^{-/-}\) mice (shaded bars). Effects of DO11.10 TCR transgene (DO11) and targeted mutation of MHC class II (Ia\(^b\)) are compared with control animals derived from the same genetic background. (DO11 \(^+\) and Ia\(^b\), respective) CYD \(^+\) and CD8 \(^+\) T cell subsets were determined by flow cytometry. DO11 \(^+\) mice lacked significant numbers of CD8 \(^+\) cells and Ia\(^b\) mice lacked significant CD4 \(^+\) cells, regardless of CD25 genotype. Mean and SD are given, reflecting analysis of 5–17 animals ages 5–12 wk for each group.](http://www.jimmunol.org/Downloadedfrom)
abolished in athymic mice lacking IL-2. We studied the effects of the Iaβ2/2 mutation and the DO11.10 TCR transgene and on serum IgM and IgG1 levels in CD252/2 mice (Fig. 2). As expected, CD25 was required to regulate IgG1, but not IgM levels Iaβ1mice. This effect was abolished in Iaβ2/2 mice. Despite the limited TCR repertoire, DO11.10 CD252/2 mice had IgG1 levels 10-fold higher than the CD251/2 littermates. Taken together, these results indicate that hypersecretion of Igs in the absence of CD25 occurs as a result of deregulated CD4+ T cells. This effect may be nonspecific, inasmuch as a diverse TCR repertoire was not required.

Allelic exclusion is maintained in expanded CD4+ DO11.10 T cells in CD25−/− mice

Because suppression of endogenous TCR expression by the DO11.10 TCR transgene is incomplete, the lymphoid expansion

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Vβ8+</th>
<th>% KJ1-26+</th>
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<tbody>
<tr>
<td>CD25+/-</td>
<td>99 ± 0.2 (n = 9)</td>
<td>82 ± 2.9 (n = 13)</td>
</tr>
<tr>
<td>CD25−/-</td>
<td>98 ± 1.0 (n = 4)</td>
<td>82 ± 2.4 (n = 15)</td>
</tr>
</tbody>
</table>

* Lymph node cells from mice 4 to 6 wk old were analyzed by flow cytometry, and the percentage of CD4+ cells expressing either Vβ8 or KJ1-26 was assessed. Mean values ± SD are given.

CD25 was required to regulate IgG1, but not IgM levels Iaβ+ mice. This effect was abolished in Iaβ−/− mice. Despite the limited TCR repertoire, DO11.10 CD25−/− mice had IgG1 levels 10-fold higher than the CD25+/- littermates. Taken together, these results indicate that hypersecretion of Igs in the absence of CD25 occurs as a result of deregulated CD4+ T cells. This effect may be nonspecific, inasmuch as a diverse TCR repertoire was not required.

Allelic exclusion is maintained in expanded CD4+ DO11.10 T cells in CD25−/− mice

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**FIGURE 2.** CD25-dependent B cell regulation requires CD4+ T cells but not a diverse TCR repertoire. Serum IgM and IgG1 levels were determined by ELISA in 4–11 individuals from each group and expressed as mean ± SD. Open bars, CD25+/2 mice; shaded bars, CD25−/− mice.

**FIGURE 3.** Activation of peripheral CD4+ T cells in CD25−/− mice occurs in subsets with both diverse and restricted TCR repertoire diversity. Lymph node cells from mice of the indicated genotypes were analyzed by flow cytometry for the indicated surface markers, which are plotted on a logarithmic scale. Data were gated on either total CD4+ T cells or on CD4+ T cells with either high or absent KJ1-26 expression. Data shown are representative of 3–10 individuals for each genotype.

**Table 1.** Lymphoid expansion in DO11.10 CD25−/− mice is not selective for cells lacking transgene expression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Vβ8+</th>
<th>% KJ1-26+</th>
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<tbody>
<tr>
<td>CD25+/-</td>
<td>99 ± 0.2 (n = 9)</td>
<td>82 ± 2.9 (n = 13)</td>
</tr>
<tr>
<td>CD25−/-</td>
<td>98 ± 1.0 (n = 4)</td>
<td>82 ± 2.4 (n = 15)</td>
</tr>
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</table>
observed in DO11.10 CD25<sup>−/−</sup> mice could reflect selective proliferation of the subset of T cells with a more diverse TCR repertoire, which would presumably retain some capacity for high-affinity recognition of environmental Ags. Expression of the DO11.10 TCR on peripheral T cells was assessed using either Abs to V<sub>j8</sub>, which recognize the transgenic TCRβ chain, or the KJ1-26 anti-clonotypic Ab, which requires expression of both the transgenic TCR α and β-chains (Table I). No differences were noted in the proportions of CD4<sup>+</sup> cells expressing these markers in CD25<sup>+/−</sup> and CD25<sup>−/−</sup> mice, demonstrating that the T cell expansion in DO11.10 CD25<sup>−/−</sup> mice was not due to selection of cells lacking OVA specificity. Peripheral T cells in CD25<sup>−/−</sup> mice characteristically display an activated phenotype, including a large proportion of cells expressing high levels of CD44, low levels of CD62L, as well as up-regulation of CD69 (20, 29). A similar phenotype was observed in the CD4<sup>+</sup> T cell population in DO11.10 CD25<sup>−/−</sup> mice (Fig. 3). Comparison of CD4<sup>+</sup> T cells expressing or lacking the transgenic TCR clonotypic marker KJ1-26 revealed that both subsets displayed an equivalent phenotype in CD25<sup>−/−</sup> mice, suggesting that differences in TCR repertoire diversity between these subsets were irrelevant to the regulatory role of CD25. We next considered whether expansion of CD25<sup>−/−</sup> T cells reflected escape from allelic exclusion of endogenous TCR. Rearrangement of endogenous TCRβ chains was compared in purified CD4<sup>+</sup> T cells from DO11.10 CD25<sup>−/−</sup> and DO11.10 CD25<sup>+/−</sup> mice. Genomic DNA was subjected to quantitative Southern blot analysis using a probe specific for the germline (unrearranged) TCRβ locus (Fig. 4). Using a probe for a nonrearranging gene to normalize for lane loading, retention of the germline TCRβ signal in T cells was compared with kidney DNA (26) and quantitated in CD25<sup>+/−</sup> and CD25<sup>−/−</sup> samples at 66 and 67%, respectively. A smaller band seen in the T cell lanes is consistent with DJ rearranged alleles, in agreement with previous studies (30). These results show that expansion of CD25<sup>−/−</sup> T cells was not selective for cells with productively rearranged endogenous TCRβ genes. Allelic exclusion of TCRα chains is much less stringent than for TCRβ. In DO11.10 mice, expression of the KJ1-26 clonotype on peripheral CD4<sup>+</sup> cells is variable and is down-regulated in cells with high expression of endogenous TCRα chains. Our observation that KJ1-26 expression is equivalently distributed among CD4<sup>+</sup> T cells in CD25<sup>−/−</sup> mice (Table I) therefore suggests that T cell expansion was not due to selection for cells expressing endogenous TCRα. We directly assessed allelic exclusion of V<sub>α2</sub> and V<sub>α8</sub> in CD4<sup>+</sup> T cells from DO11.10 mice, comparing KJ1-26- and KJ1-26-positive subsets (Table II). In CD25<sup>−/−</sup> mice, the percentage of DO11.10 CD4<sup>+</sup> T cells expressing V<sub>α2</sub> and V<sub>α8</sub> was similar to that seen in BALB/c mice, whereas the marked reduction in endogenous V<sub>α</sub> expression was observed in the CD4<sup>+</sup> KJ1-26<sup>+</sup> T cell subset. The degree of TCRα allelic exclusion was essentially the same in CD25<sup>+/−</sup> mice, indicating that T cell expansion in CD25-deficient mice was not due to selection of cells expressing these endogenous TCRα. Taken together, these data indicate that CD25-dependent regulation of CD4<sup>+</sup> T cells is equally important for T cell subsets with a highly restricted TCR specificity and those with a semidiverse repertoire.

**Deregulation of peripheral T cells in CD25<sup>−/−</sup> mice requires expression of endogenous TCRα chains**

The observation that CD25 is needed for homeostasis in T cell subsets across an increasingly restricted range of TCR diversity raises the question of whether TCR specificity is at all relevant to

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**Table II. Lymphoid expansion in CD25<sup>−/−</sup> mice does not alter allelic exclusion of endogenous Vα chains among T cells expressing the KJ1-26 clonotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; Vα2 KJ1-26&lt;sup&gt;−&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; Vα2 KJ1-26&lt;sup&gt;+&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; Vα8 KJ1-26&lt;sup&gt;−&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; Vα8 KJ1-26&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>7</td>
<td>9.9 (0.42)</td>
<td>3.1 (0.42)</td>
<td>16 (2.5)</td>
<td>3.5 (0.98)</td>
</tr>
<tr>
<td>DO11.10 CD25&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>6</td>
<td>16 (2.5)</td>
<td>3.1 (0.37)</td>
<td>3.5 (0.62)</td>
<td>0.85 (0.10)</td>
</tr>
<tr>
<td>DO11.10 CD25&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>8</td>
<td>15 (2.0)</td>
<td>3.5 (0.62)</td>
<td>3.2 (1.1)</td>
<td>0.83 (0.19)</td>
</tr>
</tbody>
</table>

*Values represent mean (SD) percentages of the indicated T cell subset.*
the expansion of T cells in CD25−/− mice. To explore this question, we bred CD25-mutant DO11.10 mice onto the Rag-2-deficient background to generate mice with only a single TCR specificity. Rag-2−/− mice on a mixed C57BL/6 and 129 background were intercrossed and subsequently backcrossed for three to four generations with DO11.10 CD25+/+ mice, selecting for animals which were homozygous for H-2d. Rag-2−/− DO11.10 H-2d/bi mice were then generated with either the CD25+/+ or CD25−/−-genotype. As expected, >90% of lymph node cells were CD4+ and all had uniformly high KJ1-26 expression (Fig. 3). No differences were observed in the number of peripheral CD4+ KJ1-26− T cells in CD25−/− vs CD25+/+ mice: the CD25−/− group (n = 6) had 1.9 ± 0.41 x 10^6 cells (mean ± SD), whereas CD25+/− littersmates (n = 5) had 2.8 ± 1.2 x 10^6 cells (differences not statistically significant). Analysis of surface markers (Fig. 3) indicated that CD4+ KJ1-26− T cells in both the CD25+/+ and CD25−/− groups had a resting, naive phenotype. These results show that mutation of Rag-2 suppressed the expanded and activated T cell phenotype in CD25−/− mice, suggesting that a measure of TCR diversity, presumably reflecting the capacity of some T cells to react with environmental Ags, is a required element for the expanded T cell phenotype.

**Ag responses in DO11.10 CD25-mutant mice**

Signals from the IL-2R have been implicated in both Ag-induced T cell expansion and activation-induced cell death (1, 9, 16–18, 31). In DO11.10 mice, as with other TCR-transgenic mice, immunization with antigenic peptide generates a biphasic response. T cell proliferation and expansion is followed by activation-induced apoptosis (28, 32, 33). We tested the role of the high-affinity IL-2R in this model system, comparing DO11.10 mice which were either homozygous or heterozygous for the CD25 null mutation. Three days after s.c. immunization with OVA peptide, CD25+/+ animals exhibited expansion of peripheral transgenic T cells (Fig. 5). In CD25−/− mice, a significant change in T cell numbers was not observed, although the wide variation in the size of expanded lymph node in CD25−/− mice limits interpretation of this result. To circumvent this problem, and because the hypertrophic lymph node tissue in CD25-deficient mice might influence responses to Ag in vivo, we utilized the adoptive transfer system described by Kearney et al. (28) to further evaluate the responses of OVA-specific DO11.10 T cells in vivo in the context of a normal lymphoid compartment. By ameliorating the abnormally high percentage of T cells responding to a given Ag, T cell responses appear to behave in a more physiologic manner using this system. As shown in Fig. 6, T cell expansion at day 3 after immunization was partially impaired in DO11.10 CD25−/− mice, suggesting that during the early phases of Ag responses in vivo, IL-2R signals contribute to expansion of CD4+ T cells.

Cell death is a prominent outcome in the later stages of immune responses, as the vast majority of cells generated in response to Ag have a short life span (34). T cell death is prominent following continued or recurrent TCR stimulation and occurs in the context of cell cycle transit (35, 36). We investigated peripheral deletion of CD4+ T cells in DO11.10 mice 8 days after OVA administration. As shown in Fig. 5, peripheral T cell deletion was efficient in both the CD25+/+ and CD25−/− backgrounds, despite the fact that unimmunized CD25−/− mice had an expanded lymphoid compartment. Deletion of transgenic T cells in response to OVA was accompanied by an increase in apoptotic cells in the lymph node, which was comparable in the CD25+/+ and CD25−/− backgrounds (Fig. 7). We observed a contraction of CD4+ KJ1-26+ T cells adoptively transferred into BALB/c mice (Fig. 6), with similar numbers of CD25+/+ and CD25−/− cells remaining 8 days after OVA immunization. However, given that the maximal expansion of CD25−/− T cells was only half that observed for CD25+/+ controls, CD25−/− T cells underwent a proportionately lesser degree of clonal contraction. Taken together, these data suggest that high-affinity IL-2R signals are not required for efficient CD4+ T cell death in vivo following an encounter with cognate Ag.

**Discussion**

IL-2R signals are complex at a cellular level and may influence several alternative cell fates following T cell activation, including...
Peripheral lymphoid homeostasis is mediated by effects on T cells. Not all mice defective in IL-2R signaling have demonstrated that peripheral lymphoid homeostasis is mediated by effects on T cells. Expansion of the lymphoid compartment and autoimmune disease are abrogated in IL-2−/− mice but are unaffected in IL-2−/−JH−/− mice which lack B cells (22, 39). It has been postulated that this pathologic changes in IL-2−/− mice are mediated by activated CD4+ T cells (40). Our data clarify and extend these observations as follows. High-affinity IL-2R signals appear to regulate the size of the CD4+ and CD8+ T cell compartments independently, since CD8+ T cell expansion in CD25−/− mice was unaffected in the Jαβ-null background where CD4+ T cells are deficient and because CD4+ T cell expansion was intact in CD25−/−DO11.10 mice which have few CD8+ T cells. In the B cell compartment, defective IL-2R signals lead to marked elevations in serum quantities of Ig isotypes characteristic of secondary immune responses. Our data show that this phenotype is independent on the CD4+ T cell subset and suggest that hypersecretion of certain Ig isotypes is an indirect effect of expanded and activated CD4+ T cells, rather than reflecting an intrinsic abnormality of B cell function. The fact that the CD4+ T cell compartment in DO11.10 CD25−/− mice was sufficient to drive Ig hypersecretion in vivo, despite a severely limited TCR repertoire, further suggests that B cells were activated outside the context of Ag-specific immune responses.

Lymphoid homeostasis involves a balance between cells generated within or entering the peripheral compartment and cell death. IL-2R signals promote cellular proliferation in vitro (9, 16, 18); however, experiments testing whether IL-2R signals play a central role in mediating T cell expansion following Ag stimulation in vivo have given mixed results. CD4+ T cell expansion in response to superantigens is intact in mice lacking either CD25 or IL-2 (20, 23). In young IL-2-deficient mice, immune responses, including antiviral CTL or T cell-dependent Ab responses, are normal or mildly impaired, although the CTL defect is severe in older animals (41). A more detailed accounting of CTL expansion in response to lymphocytic choriomeningitis infection found a substantial reduction in IL-2-deficient mice (31). Interpretation of these studies is complicated by the fact that the peripheral lymphoid compartment in mice with defective IL-2R signaling is markedly abnormal, and this factor is difficult to separate from cell-autonomous effects of IL-2R signals on T cells stimulated by Ag. Our data show that when placed in the context of a normal lymphoid compartment, CD25-deficient T cells exhibit a significant reduction in Ag-induced expansion, indicating that although IL-2R signals are not required for T cell proliferation in vivo, they do contribute to this process. In studies similar to those presented here, Khoruts et al. (42) reported that Ag-induced expansion of adaptively transferred DO11.10 IL-2−/− T cells was normal or higher than that of wild type. However, because IL-2−/− T cells are sensitive to IL-2 (19), a contribution of paracrine activity from IL-2 generated by recipient T cells could not be excluded. Taken together, the existing data suggest that T cell expansion during immune responses is mediated by multiple, partially redundant growth factors in vivo, and the relative contribution of IL-2R signals may vary with the particular conditions of individual responses.

It has been postulated that the homeostatic defect in mice lacking CD25 or IL-2 reflects a role for IL-2R signals in deletion of peripheral T cells following activation by cognate Ags (9, 18, 20, 23). Our data suggest that for Ag-specific T cell responses in vivo, high-affinity IL-2R signals are not required for activation-induced apoptosis in the periphery. These data appear to conflict with the observation that peripheral deletion of T cells in response to immunization with bacterial superantigens is impaired in mice lacking CD25, IL-2, or γc (20, 23, 43), as well as the finding that Fas-dependent apoptosis, which is involved in a subset of Ag-induced peripheral T cell deletion events, is impaired in activated CD25−/− or IL-2−/− T cells (23, 33, 44, 45). However, mice lacking IL-2R have normal superantigen-mediated deletion of peripheral T cells, suggesting that IL-2R signals are not fundamentally required for this process. Like the situation with T cell expansion, the activity of IL-2R signals in promoting Ag-induced T cell death appears to be redundant with other cytokines that promote cell cycle progression, including IL-4, IL-7, and IL-15 (35, 45, 46). The effects observed may therefore vary depending on characteristics of the Ag and dose used. Altogether, investigations addressing the consequences of defective IL-2R signals for Ag-dependent T cell responses in vivo do not explain the phenotype of lymphoid expansion in gene-deficient mice.

Because the DO11.10 TCR transgene is specific for a high-affinity Ag not normally encountered in the environment of laboratory mice, we predicted that transgenic CD4+ T cells would be unaffected by the absence of CD25 until Ag was administered experimentally. Unexpectedly, we found that CD4+ T cells in DO11.10 CD25−/− mice exhibited peripheral expansion which was equivalent to nontransgenic CD25−/− mice, both in magnitude and in age of onset. This finding suggested that the diversity of the TCR repertoire, or perhaps TCR specificity itself, was unimportant in CD25-dependent homeostasis. Because of leakiness in allelic exclusion, endogenous α chains are expressed in a subset of T cells in DO11.10-transgenic mice. This creates subpopulations with TCR diversity ranging from semidiverse (endogenous TCRα paired with transgenic TCRβ) to highly restricted (transgenic TCRαβ only). This spectrum of diversity is paralleled by expression of the KJ1-26 clonotype: endogenous α-chains are present in proportions similar to normal BALB/c mice in CD4+ T cells with low or absent KJ1-26 expression, whereas they are markedly reduced within the KJ1-26(B) T cell subset. If CD4+ T cell expansion in DO11.10 CD25−/− mice was due to impaired cell death following stimulation by a variety of environmental Ags, then preferential expansion of cells with a broader T cell repertoire would be predicted, leading to a skewing in favor of cells lacking KJ1-26, or otherwise escaping allelic exclusion. Instead, we found that the expanded T cell population had an identical composition with respect to KJ1-26 expression, Vβ8 expression, as well as allelic exclusion of Vα3 and Vα8 within the KJ1-26(B) subset. In parallel with these results, the activated T cell phenotype which accompanies lymphoid expansion in CD25−/− mice was identical in KJ1-26+ and KJ1-26− T cell subsets. The latter observation differs from DO11.10 mice lacking γc, where the activated phenotype of peripheral T cells was restricted to the KJ1-26− subset (47). These differences presumably reflect the requirement for γc in multiple cytokine receptors, which may be involved at several levels in the positive and negative regulation of the peripheral T cell compartment.
The observation that CD25 is required for peripheral homeostasis of T cells with restricted specificity suggests that regulatory signals may operate independent of exposure to cognate Ag. However, there is an apparent contradiction in that CD25 was not needed for T cell homeostasis in DO11.10 Rag-2\(^-/-\) CD25\(^-/-\) mice, which have unispecific TCR repertoire. One potential explanation is that all of the expanded T cells in DO11.10 CD25\(^-/-\) mice express endogenous TCRs and were stimulated by cognate Ags from the environment. Because we were not able to assess expression of all possible TCR \(\alpha\)-chains, this explanation remains a formal possibility but is strongly discounted by the observation that allelic exclusion of V\(\alpha\)3 and V\(\alpha\)8 in DO11.10\(^+\) CD25\(^-/-\) T cells was identical to that in control mice. A more likely explanation is that many if not most of the individual T cells were stimulated by signals other than cognate interactions between TCR and high-affinity Ags. Such signals could be generated indirectly as a result of Ag-specific activation of the subset of T cells bearing a more diverse TCR repertoire, thus representing a bystander effect. This interpretation may also apply to CD25-dependent regulation of CD8\(^+\) T cells in Ia\(\beta\)\(^-/-\) mice, which are also severely deficient in their ability to respond to exogenous Ags (25). Following this argument, our data suggest that a major function of high-affinity IL-2R signals in vivo may be to suppress activation and expansion of T cells by signals of low specificity, including bystander activation.

Immune stimuli efficiently activate T cells through high-specificity interactions. However, such responses likely include a spectrum of T cell activation events at varying levels of TCR affinity, many of which are presumably nonproductive with respect to immune effector responses or possibly even detrimental. Production of IL-2 is limited to T cells activated under conditions of high specificity, in that proper costimulatory signals are required (48, 49). In contrast, CD25 expression, which represents a critical link in cellular responsiveness to IL-2, occurs in response to a much broader range of stimuli: including TCR signals outside the context of costimulation as well as non-TCR signals such as IL-1 (9, 11). IL-2R signals may promote cell cycle progression in both of these situations, leading to increased proliferation in the case of high-affinity TCR interactions and conditions of optimal costimulation and promoting apoptosis in the case of poorly constituted TCR-Ag-MHC interactions. In this manner, high-affinity IL-2R signals may assist in narrowing the specificity of T cell activation and controlling the overall magnitude of lymphoid expansion.

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References


